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(54) Title: SCAVENGER RECEPTOR PROTEIN AND ANTIBODY THERETO

(57) Abstract

A substantially pure receptor protein capable of binding acetylated low density lipoprotein and oxidized low density lipoprotein is disclosed herein. This protein is characterized by having a molecular weight of about 220,000 daltons, and an affinity for oxidized low density lipoprotein and acetylated low density lipoprotein. Further, it is an integral membrane protein which includes a collagen domain. Proteins having an affinity for the receptor protein as well as DNA sequences encoding at least a portion of the receptor protein are also disclosed herein. In addition, devices for purification purposes and methods for detecting atherosclerotic plaques are described, both of which utilize the receptor protein or binding proteins thereto.

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SCAVENGER RECEPTOR PROTEIN AND ANTIBODY THERETO

Background of the Invention

The technical field of this invention is
devices and methods for the diagnosis and treatment
of atherosclerosis, and, in particular, devices and
methods utilizing receptor proteins associated with
atherosclerotic plaques and/or monoclonal antibodies
thereto.

Atherosclerosis is a disease which causes
the thickening and hardening of the arteries,
particularly of the larger arteries. It is
characterized by raised fibrous plaques or lesions
within the arterial lumen which form as the result of
the deposition of plasma cholesterol and low density
lipoproteins (LDL).

Key cellular components of the
atherosclerotic plaque are foam cells which are
derived from phagocytic macrophages of monocytic
origin. Macrophages and foam cells have surface
receptor proteins which have an affinity for

circulating LDL, and which are directly responsible
for the receptor-mediated ingestion, or endocytosis,
of such material.

These cells also have a receptor-mediated
ability to bind and endocytose chemically modified
LDL, such as acetylated-LDL (Ac-LDL), acetoacetylated
LDL (AcAc-LDL), and oxidized LDL (Ox-LDL), as well as
a limited range of other negatively charged
macromolecules. This receptor activity has been
found to be inducible in monocytes and macrophages
under certain conditions, and has been implicated in
the formation of foam cells, and hence of
atherosclerotic plaques (Goldstein et al. vol. 76,
Proc. Natl. Acad. Sci. (USA), pp. 333-337 (1980);
Brown et al. vol. 52, Ann. Rev. Biochem., pp. 222-226
(1983)).

The injuries or deformities of the arterial
lumen caused by the plaque and associated deposits
result in occluded blood flow, and ultimately in a
number of related conditions if left untreated, such
as, angina, cerebral ischemia, renal hypertension,
ischemic heart disease, stroke, and diseases of other
organs. Coronary atherosclerosis is still the
leading cause of death in the United States and in
other industrially advanced countries.

Unfortunately, there are no existing
diagnostic methods which can reliably detect these
diseases. The early stages of atherosclerosis and
related vascular diseases often have no clinically
manifestations. Because lifestyle changes, drug
th rapy, and ther means exist for delaying or

reducing vascular occlusion or the str ss s on
various body organs which result from ath rosclerotic
lesions, the early detection of atheromatous plaques
would be of considerable value in permitting
preventive intervention at a time when it can be most
effective.

Arteriography is the conventional approach
to diagnosing advanced vascular disease. This
procedure involves the imaging of obstructions in the
arteries via catheterization and the injection into
the bloodstream of radioopaque substances. However,
significant morbidity can result from this procedure
due to the increased chances of infection,
perforation of the artery, arrhythmia, stroke, and
infarction. Because of the risks involved, ·
arteriograms typically are reserved for individuals
with advanced or acute atherosclerotic disease,
ruling out this method for preventative therapy.

A variety of less invasive techniques for
the diagnosis of vascular injury and disease have
been used. These techniques include plethysmography,
thermography, and ultrasonic scanning. (For a review
of these procedures, see Lees and Myers, Vol. 27,
Adv. Int. Med., pp. 475-509 (1982)). However, none
of these imaging techniques have achieved clinical
acceptance.

Other non-invasive approaches to the
diagnosis of vascular injury include the
administration of injectible, detectible agents to
the vascular system of a patient which are capable of
recognizing, binding to, and/or being internalized

by, atherosclerotic lesions. Such procedures include
the administration of hematoporphyrin (as described
in U.S. 4,577,636, issued to Spears), monoclonal
antibodies (EPO 85402359.5 issued to Takano), or
magnetic particles capable of being inductively
heated (as described in U.S. 4,359,453 issued to
Gordon).

Because LDL is known to be deposited in
atherosclerotic plaques, methods employing labelled
LDL have been utilized to target plaques (see, for
example, U.S. Patent Nos. 4,647,445 and 4,660,563
issued to Lees). A disadvantage to these methods is
that several days are typically required to isolate
LDL from the patient's blood and to label them.
Often, such a delay in diagnosis and subsequent
treatment is detrimental for critically ill
patients. Furthermore, an additional risk of viral
infection can arise if donor blood is employed as an
LDL source.

Consequently, there exists a need for better
non-invasive techniques and reagents capable of
detecting, mapping, and treating early,
non-stenosing, non-flow-disturbing atherosclerotic
arterial lesions.

Accordingly, it is an object of the present
invention to provide methods for the early detection
of atherosclerosis and other related vascular
diseases. It is also an object of the present
invention to identify a component of the arterial
plaque which is key in detecting and mapping an
abnormal region of th vascular system, or a region

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predisposed to atherosclerosis. Yet another obj ct
of th invention is to provide a d tection method
which is non-invasive. A further object of the
present invention is to provide devices and methods
for locating and quantitating the extent of arterial
plaque build up. Another object is to provide a
method for treating atherosclerosis.

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Summary of the Invention

Receptor proteins, antibodies thereto, and methods and devices utilizing such receptor proteins and antibodies are disclosed herein for the detection and treatment of atherosclerosis. A new receptor protein has been discovered which is capable of binding chemically modified forms of LDL such as acetyl-LDL (Ac-LDL). This receptor protein is found on the surface of macrophages and plaque-forming components. The term "plaque-forming component" is used herein to encompass cells associated with the plaque and/or involved in its genesis such as foam cells, macrophages, and monocytes.

The new receptor protein, referred to hereinafter as the "scavenger receptor protein", or the "receptor protein" has an apparent molecular weight on SDS-polyacrylamide gels of about 220,000 daltons (220 kD), and a binding affinity for Ac-LDL of about 0.5 micrograms per milliliter. It is further characterized as being a trimer of subunits, each of which having an apparent molecular weight on SDS-polyacrylamide gels of about 77 kD, and as including an asparagine (Asn)-linked carbohydrate chain.

In addition, it appears that this novel receptor protein includes not only a region or domain which spans the membrane followed by an alpha-helical triple coiled coil domain, but also an extracellular collagen domain. The term "collagen domain" is used herein to encompass a region of a polypeptide which is substantially analogous to that of collagen or an

analog or portion thereof. This collagen domain is found in each of the three subunits.

The present invention includes a substantially pure scavenger receptor protein and an active fragment, analog, and derivative thereof. One embodiment of the invention includes a polypeptide having an amino acid sequence sufficiently duplicative of the amino acid sequence of a subunit of the scavenger receptor protein such that said polypeptide, when trimerized with two of said polypeptides or with two subunits of the scavenger receptor protein, binds chemically modified forms of LDL. The invention also includes DNA sequences encoding active fragments, derivatives, and subunits thereof.

Further, invention includes a binding reagent with an affinity for the scavenger receptor protein or specific portion thereof. In a preferred embodiment, a monoclonal antibody is disclosed as a binding agent with a specific affinity for the scavenger receptor protein. Such a device could be used to bind various molecules for which the receptor proteins, or fragments or analogs thereof, have affinity.

In one aspect of the invention, a device for assay or purification purposes is disclosed which includes a plurality of scavenger receptor proteins, or fragments or analogs thereof, linked to a support. The invention also includes a device for assay or purification which includes a plurality of

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binding reagents with an affinity for the scavenger
receptor protein, linked to a support.

In other aspects of the invention, methods
utilizing the diagnostic properties of the scavenger
receptor protein and the binding reagent thereto are
also disclosed. For example, a method of detecting
an atherosclerotic plaque is described. In this
method, the binding reagent is coupled to a labelling
means to form a conjugate. The conjugate is then
administered to the vascular system of a subject
where it is allowed to bind to the scavenger receptor
protein associated with a plaque and/or a
plaque-forming component. The presence and location
of the labelling means in the vascular system of the
subject is then determined, as they are indicative of
the presence of an atherosclerotic plaque.

The present invention has therapeutic
utility as well. For example, one embodiment
includes a method of treating atherosclerosis by the
selective delivery of a therapeutic agent to a
plaque. In this application, a therapeutic agent is
coupled to a binding agent having a specific affinity
for the scavenger receptor protein, thereby forming a
conjugate therapeutic agent. The conjugate is then
administered to the vascular system of the subject
where it binds to the receptor protein and delivers
the therapeutic agent to the plaque or plaque-forming
component.

In further aspects of the invention, methods
utilizing antagonists of the scavenger receptor

protein are disclosed for treatment of vascular
dis ase.

The invention will next be described in
connection with certain illustrated embodiments.
However, it should be clear that various
modifications, additions, and subtractions can be
made without departing from the spirit or scope of
the invention.

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Description of the Drawings

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself may be more fully understood from the following description when read together with the accompanying drawings in which:

FIG. 1 is a photographic representation of a non-reducing SDS gel of the partially purified scavenger receptor protein which has been silver stained (A); ligand blotted (B); and immunoblotted (C);

FIG. 2 is a schematic representation of the scavenger receptor protein and its domains;

FIGs. 3A-C are a schematic representation of a first representative nucleic acid sequence and corresponding amino acid sequence of a portion of the protein illustrated in FIG. 2;

FIGs. 4A-D are a schematic representation of a composite nucleic acid sequence and the corresponding amino acid sequence of another overlapping portion of the protein;

FIG. 5 is a photographic representation of the scavenger receptor protein which has been subjected to immunoprecipitation, non-reducing SDS-PAGE and then to autoradiography (A) or ligand blotting (B), or to immunoaffinity purification followed by non-reducing SDS-PAGE and silver staining (C);

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FIG. 6 is a photographic representation of immunoprecipitated 77 kD subunit of the receptor protein which has been digested with sialidase (B) or endoglycosidase F (C) prior to reducing SDS-PAGE;

FIG. 7 is a photographic representation of the immunohistochemical localization of the scavenger receptor protein in bovine liver incubated with (A) control antibody, or (B, C) IgG-D1;

FIG. 8 is a photographic representation of (A) a fluorescence photograph demonstrating the ability of macrophages to take up fluorescent labelled acetyl-LDL in the absence (1) or presence (2) of excess unlabelled acetyl-LDL; and (B) the susceptibility of the scavenger receptor protein to trypsin digestion;

FIG. 9 is a photographic representation of a ligand blot demonstrating the coinduction of the scavenger receptor protein and cellular scavenger receptor-mediated endocytosis in human monocyte (PMA-)/macrophage (PMA+) cell lines;

FIG. 10 is a graphic representation of the binding affinity (A) and specificity (B) of acetyl-LDL binding to the affinity purified receptor protein;

FIG. 11 is a graphic representation of the ability of different concentrations of a number of competitors to inhibit Ac-LDL receptor activity;

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FIG. 12 is a graphic r presentation of the ability of one concentration of different competitors to inhibit Ac-LDL receptor activity;

FIG. 13 is a photographic representation of an autoradiogram of a Northern blot using RNA isolated from various cell and tissue types and a DNA probe encoding the collagen domain; and

FIGs. 14A-D are a schematic representation of a representative nucleic acid sequence and corresponding amino acid sequence of a subunit of the human scavenger receptor protein.

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Detailed Description

     The scavenger receptor protein is
differentially expressed in various tissues. It can
be isolated from various organs and, in particular,
from mammalian liver and lung specimens by
established purification methods. These procedures
include, for example, differential centrifugation,
detergent extraction of isolated membrane fractions,
ion exchange or exclusion chromatography, and gel
chromatography.

     Alternatively, purification methods
employing an antibody which has a specific affinity
for the receptor protein may be employed. For
example, the receptor protein may be purified by
affinity chromatographic methods in which the
antibody is bound to an immobilized matrix or support
material in a column; the receptor protein may also
be immunoprecipitated from a heterogeneous solution
using an anti-receptor protein antibody. Using this
methodology, the receptor protein may also be
isolated from mammalian foam cells and plaque-forming
components such as macrophages .

     Using these isolation methods, the scavenger
receptor protein has been purified to at least near
homogeniety. The protein has an apparent molecular
weight on SDS-polyacrylamide gels of about 220 kD
(FIG. 1A), and a binding affinity for Ac-LDL. It
consists of three seemingly identical subunits, each
of which has an apparent molecular weight of about 77
kD (FIGs. 1 and 2), and each apparently having a
number of differ nt domains (i.e., Cys-rich domain,

collagen domain, asparagine-linked sugar domain, transmembrane domain, and cytoplasmic domain) which are depicted in FIG. 2.

As detailed below, a major portion of the amino acid sequence of the protein has been derived from the nucleic acid sequence of a gene encoding the protein. However, because more than one nucleotide triplet (codon) can encode a single amino acid, a number of different nucleotide sequences can encode a single protein. Hence, the peptide fragment disclosed herein may be encoded by nucleic acid sequences which are functionally equivalent to the one shown above, and which may also be prepared by known synthetic procedures. Accordingly, the invention includes such functionally equivalent nucleotide sequences. In addition, one skilled in the art, knowing the amino acid sequence of the receptor protein, could synthetically or biosynthetically prepare a functionally equivalent analog of the receptor protein of the invention having substantially the same biological activity. In particular, fragments of the protein, especially portions of the extracellular domain, can be obtained for the disclosures herein without undue experimentation.

Thus, the scope of the invention includes the amino acid sequences and corresponding nucleic acid sequences depicted herein, as well as all functionally equivalent amino acid sequences (and corresponding nucleic acid sequences) for molecules with substantially the same scavenger receptor protein biol gical activiti s. For example, one

embodiment of the invention includes a polypeptide
having an amino acid sequence sufficiently
duplicative of the amino acid sequence of a subunit
of the scavenger receptor protein such that the
polypeptide, when trimerized with two other like
polypeptides or with two subunits of the scavenger
receptor protein, bind chemically modified forms of
LDL..

Furthermore, those skilled in the art of
recombinant DNA technology can use a nucleic acid
sequence encoding the receptor protein or a fragment
thereof to produce a protein, fragment thereof, or an
analog thereof in an appropriate microbial or
mammalian host cell. For example, the sequence may
be inserted into an expression system such as a
vector which is suitable for transforming or
transfecting a eucaryotic (yeast or mammalian) or
prokaryotic (bacterial) host cell. These standard
procedures have been followed to produce well-known
proteins such as insulin, interferons, human growth
hormone, and the like. Similar procedures, or
obvious modifications thereof, can also be employed
to prepare the scavenger receptor protein, and
fragments or analogs thereof in accord with the
subject invention.

Using such recombinant DNA methodologies,
several clones were prepared which encode at least
part of the scavenger receptor protein. Clone 3
encodes at least the membrane spanning (TM) domain
and the collagen (Col) domain, as well as an
asparagine (Asn)-linked sugar domain (N-linked sugar)
including a numb r of potential sugar linkage sites.

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The nucleic acid sequences and corresponding amino
acid sequence of clone 3 is shown in FIGs. 3A-C
wherein the first underlined amino acid sequence is
that of the membrane spanning domain, the second
underlined sequence is that of the collagen domain,
and overlined residues 82-84, 101-103, 142-145,
183-186, 220-222, 248-250, 266-268 are the potential
sugar linkage sites within the Asn-linked sugar
domain.

Clones 7, 12, and 13 include some or all of
these domains as well as at least a portion of a
cysteine rich domain. A composite nucleic acid
sequence and corresponding amino acid sequence of
these clones is shown in FIGs. 4A-D.

FIGs. 14A and 14B show a representative
nucleic acid sequence and corresponding amino acid
sequence of at least part of the human scavenger
receptor protein determined using similar DNA
methodologies.

TABLE 1 provides the abbreviations for the
amino acids shown in FIGs. 3A-C, 4A-D, and 14A-D.

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TABLE 1

amino acid	abbreviations		amino acid	abbreviations	
Alanine	Ala	A	Glycine	Gly	G
Valine	Val	V	Serine	Ser	S
Leucine	Leu	L	Threonine	Thr	T
Isoleucine	Ile	I	Cysteine	Cys	C
Proline	Pro	P	Tyrosine	Tyr	Y
Phenylalanine	Phe	F	Asparagine	Asn	N
Tryptophan	Trp	W	Glutamine	Gln	Q
Methionine	Met	M	Aspartic Acid	Asp	D
Lysine	Lys	K	Glutamic Acid	Glu	E
Argenine	Arg	R	Histidine	His	H

TABLE 2 illustrates the location of the various domains within the amino acid sequence of the scavenger receptor protein.

TABLE 2

amino acid #	Domain	Estimated Mass*
1 - 50	Cytoplasmic	5,740
51 - 76	Transmembrane	2,766
77 - 271	Asn-linked Sugar	22,480
272 - 343	Collagen	6,795
341 - 453	Cys-Rich	12,275
		50,056 total

*based on the molecular weight of the individual amino acids

Each subunit has at least one asparagine (Asn)-linked carbohydrate chain determined by treatment with various deglycosylation enzymes (FIG. 6). The assignment of the Asn-linked sugar domain is based on the presence of potential Asn-linked sugar attachment sites from the cDNA sequence shown in FIGs. 3A-C, and the finding that several of these show no detectable Asn in the protein sequence, suggesting that the Asn groups are indeed glycosylated. By analysis of its amino acid sequence, this domain appears to have an alpha-helical triple coiled coil structure.

The assignment of residues 341-453 as a Cys-rich domain is based on the fact that it contains multiple Cys amino acids. In addition, because this domain is the most distal external domain, and because several other known cell surface receptors have Cys-rich ligand binding domains, this domain may also serve as a ligand binding site. However, since the collagen domain is positively charged and since the ligands are often negatively charged, the collagen domain alternatively or additionally may participate in ligand binding. The same may be true for a portion or all of the alpha helical triple coiled coli in the Asn-linked sugar domain.

The membrane spanning domain of the scavenger receptor protein includes an amino acid sequence such as:

Thr-Ala-Leu-Ile-Thr-Leu-Thy-Leu-Ile-Val-Phe-
Val-Val-Leu-Val-Pro-Ile-Ile-Gly-Ile-Val-Ala-
Ala-Gln-Leu-Leu.

The collagen domain includes an amino acid sequence such as:

Gly-Pro-Pro-Gly-Pro-Pro-Gly-Glu-Lys-Gly-Asp-
Arg-Gly-Pro-Pro-Gly-Gln-Asn-Gly-Ile-Pro-Gly-
Phe-Pro-Gly-Leu-Ile-Gly-Thr-Pro-Gly-Leu-Lys-
Gly-Asp-Arg-Gly-Ile-Ser-Gly-Leu-Pro-Gly-Val-
Arg-Gly-Phe-Pro-Gly-Pro-Met-Gly-Lys-Thr-Gly-
Lys-Pro-Gly-Leu-Asn-Gly-Gln-Lys-Gly-Glu-Lys-
Gly-Ser-Gly.

The finding of a collagen-like domain (collagen domain) is consistent with the fact that the scavenger receptor is a trimer. Collagen domains are known to form triple helical trimers in those molecules which have such domains (e.g., collagens, Clq, acetylcholine esterase, serum mannose binding proteins, and lung surfactant protein). This is also true of the alpha helical triple coiled coil in the Asn-linked sugar domain.

The proposed orientation of the receptor shown in FIG. 2 (N-terminus inside, C-terminus outside) is based on the following: 1) there is only one membrane spanning domain; 2) there is no N-terminal signal sequence (presumably, the transmembrane domain is also the signal for membrane insertion); 3) the Asn-linked sugar domain and the collagen domain are expected to be extracellular since the domains have never been found to be cytoplasmic on other proteins); and 4) the charged amino acids surrounding the transmembrane domain have a polarity consistent with the protein being N-terminus in, C-terminus out.

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The scavenger receptor protein, having several extracellular domains, allows for the design of reagents which recognize and bind to a particular domain or portion thereof, perhaps thereby modifying the activity of the receptor. Such reagents include, for example, various drugs and binding molecules. Binding proteins which have an affinity for the receptor protein include, for example antibodies, and preferably, monoclonal antibodies. Antisera raised to the purified scavenger receptor protein or a portion thereof, or antibodies purified from the antisera, can be prepared using well known protocols including injection of the purified protein or fragment, or analog thereof, preferably with Freund's adjuvent, into an appropriate mammalian host animal such as a rabbit, goat, or mouse. Preferably, however, monoclonal antibodies are prepared to the scavenger receptor protein, or active fragments of such antibodies, can be generated by applying generally known cell fusion techniques (cf. G. Kohler, C. Milstein, Vol. 6, Eur. J. Immunol., pp. 511-519 (1976); M. Shulman et al., Vol. 276, Nature, pp. 269-270 (1978)), herein incorporated by reference) to obtain a hybridoma producing the antibody, by deriving a monoclonal antibody from the hybridoma, and (optionally) by subjecting the monoclonal antibody to proteolysis to obtain an active fragment such as Fab.

For example, monoclonal antibodies can be prepared by obtaining mammalian lymphocytes (preferably mouse spleen cells), committing the lymphocytes to produce antibodies (e.g., by immunizing the mammal with the particular antigen of

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interest beforehand), fusing the lymphocytes with
myeloma (or other immortal) cells to form hybrid
cells, and then culturing a selected hybrid cell
colony in vivo or in vitro to yield antibodies which
are derived from a single clone, and therefore, are
identical in structure and specificity.

Alternatively, an antibody molecule of the
appropriate specificity, or an active analog or
fragments thereof may be produced synthetically in a
protein synthesizer, or may be produced by
recombinant means in genetically engineered cells.

Serum samples from the immunized animal can
be taken and analyzed by an enzyme linked
immunoabsorbent assay ("ELISA") or the like for
antibody reaction with the immunization agent.
Animals that exhibit antibodies titers are sacrificed
and their spleens homogenized. Alternatively, the
spleen cells can be extracted and the antibody-
secreting cells expanded in vitro by culturing with a
nutrient medium. The spleen cells are then fused
with myeloma (or other immortal) cells. The
hybridomas so produced are screened to select a cell
line producing antibodies which react with the
receptor protein. Large scale antibody production
can be obtained from such receptor protein-producing
cell lines by various techniques, including the
induction of ascites tumors (e.g., after priming with
pristane) and the purification of such antibodies
from the ascites fluid by Protein A-Sepharose
affinity chromatography.

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For a further description of general
hybridoma production methods, see, e.g., Oi and
Herzenberg, "Immunoglobulin-Producing Hybrid Cell
Lines" in Selected Methods in Cellular Immunology
(Mishell and Shiigi, Ed., W.H. Freeman & Co., pp.
351-372 (1980); and Scearce and Eisenbarth,
"Production of Monoclonal Antibodies..." in Vol. 103,
Methods In Enzymology, pp. 459-469 (1983), herein
incorporated by reference. Human antibodies (i.e.,
those obtained from human-human or human-animal
hybridoma) can be used as well as animal antibodies.
For descriptions of human hybridoma production
techniques, see, e.g., U.S. 4,451,570 issued to
Royston et al; U.S. 4,529,694 issued to Lazarus et
al.; and Zurawski et al., "Continuously Proliferating
Human Cell Lines Synthesizing Antibody of
Predetermining Specificity" in Monoclonal Antibodies
(Plenum Press, New York (1980)), also incorporated by
reference.

Active fragments can be derived from the
monoclonal antibodies disclosed herein by a number of
techniques. For example, purified monoclonal
antibodies can be cleaved with a proteolytic enzyme
such as pepsin, and then be subjected to HPLC gel
filtration. The appropriate fraction containing the
desired fragment such as, for example, an Fab
fragment, can then be collected and concentrated by
membrane filtration or the like. For further
description of general techniques for the isolation
of active fragments, see, for example, Khaw et al.,
Vol. 23, J. Nucl. Med., pp. 1011-1019 (1982), herein
incorporated by reference.

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The antibodies and fragments used herein can
be labelled with radioactive tags such as
radioisotopes by a variety of techniques. For
example, the biologically active molecules can also
be labelled with a radioisotope via conjugation with
the cyclic anhydride of diethylenetriamine
penta-acetic acid (DTPA) or bromoacetyl aminobenzyl
ethylamine diamine tetra-acidic acid (BABE)
(Hnatowich et al., Vol. 220, Science, pp. 613-615
(1983); and Meares et al., Vol. 142, Analytical
Biochemistry, pp. 68-78 (1984), incorporated by
reference).

The scavenger receptor protein and the
binding proteins of the present invention can be used
for a variety of diagnostic and therapeutic
purposes. In a simple embodiment, soluble receptor
proteins are harvested and purified from eucaryotic
cells which are preferably mammalian, or from
eucaryotic or prokaryotic cells engineered by
recombinant means to produce such proteins, and used
in both radiolabelled and unlabelled states in
competitive binding assays to test for the presence
of the receptor. The receptor protein, or fragments
or analogs thereof, binding proteins thereto can also
be fixed to inert supports for purification and assay
purposes. For example, the collagen domain of the
receptor protein can be linked to an inert support
material for uses in affinity chromatographic methods
to isolated lipids and lipid-containing substance
such as endotoxin.

Various assay techniques can be practiced
employing the reag nts disclosed herein, including

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radioimmunoassays, enzyme immunoassays, heterogeneous and homogeneous assays, enzyme linked immunoabsorbent assays ("ELISA"), and the like.

An exemplary assay for chemically modified LDL or other molecule with an affinity for the receptor protein ("analyte"), can be carried out as follows. The sample (having an unknown concentration of analyte is first contacted with a known quantity of fixed receptor protein (or analog or a portion of the protein containing the epitope), during which time the analyte in the sample becomes bound to the receptor protein. The fixed support is then treated with a known quantity of radiolabelled analyte which binds to those sites on the fixed support which were unoccupied. Excess label is then washed off, and the quantity of label remaining on the support is inversely proportional to the amount of analyte originally present in the sample.

An alternative assay for analyte can be constructed using the receptor protein fixed to a solid support such as, for example, polystyrene beads or plastic microtiter wells. Samples containing analyte would be incubated with the receptor protein for a time sufficient for binding. The support is then washed to remove any unbound analyte. To detect the bound analyte, a developing reagent consisting of monoclonal or polyclonal antibodies reactive with radiolabelled or enzyme-linked analyte can be used. Alternatively, labelled receptor protein can also be employed. (See, e.g., Roitt, Essential Immunology, (Blackwell Press pp. 137-171 (1980), incorporated

her in by reference, for further descriptions of
immunoassay techniques.

Fixation of receptor protein to solid
supports can also be useful as a means for (or
additional step in) the purification of crude
chemically modified LDL. Similarly, the fixation of
monoclonal antibodies specific for the receptor
protein can be used in purifying the receptor
protein, as well as in recognizing and isolating
cells expressing the protein.

The present invention can give rise to a
number of therapeutic agents useful in treating
various vascular disorders. One class of such
compounds includes antagonist compounds or binding
reagents which would occupy the affinity sites on the
macrophages and monocytes, blocking the binding of
chemically modified LDL, and hence, serving as an
inhibitor of foam cell development and plaque
formation. Useful antagonist compounds include
antibodies and preferably, monoclonal antibodies, (or
active fragments, analogs, and derivatives thereof)
specific for the receptor protein, itself, or
portions of the protein (i.e. the extracellular
domain) which include the chemically modified LDL
binding site. Other useful antagonists include
analogs of Ac-LDL or analytes which can bind to the
receptor protein without triggering its morphogenesis
to foam cells.

The receptor protein, or fragments thereof
containing at least an extracellular domain, can also
be useful as sequest ring agents which would remove

chemically modified LDL and other analytes from the
blood stream without effecting macrophages,
monocytes, or foam cells directly.

The receptor protein can also be useful in
designing highly specific drug delivery systems to
treat atherosclerosis. The ability of the receptor
protein of the foam cell to endocytose various
molecules can be exploited to deliver anti-viral
agents, metabolites, anti-metabolities, and other
therapeutic agents across the cytoplasmic membrane of
the foam cell in the plaque. In such instances, the
therapeutic agent would be coupled to a macromolecule
that is recognized by the receptor protein, enabling
the conjugate to become bound to, and ultimately
endocytosed, by the foam cell in the plaque. Useful
macromolecules for this purpose include Ox-LDL,
Ac-LDL, and AcAc-LDL. A synthetic or genetically
engineered macromolecule could also be employed.

An antagonist of the receptor protein may be
useful in treating vascular disease. For example, a
binding molecule such as an antibody, and more
preferably, a monoclonal antibody with an affinity
for the receptor protein can be administered to the
vascular system of a subject. This antagonist will
bind to the receptor protein found on plaque forming
components such as macrophages, foam cells, and
monocytes, thereby preventing or inhibiting such
components from contributing to vascular disease such
as developing (more) plaque. The antagonist may be
any natural, biosynthetic, or synthetic molecule
capable of binding and inhibiting the receptor
prot in.

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Th invention will next b describ d in
connection with c rtain illustrated embodiments.
However, it should be clear that various
modifications, additions and subtractions can be made
without departing from the spirit or scope of the
invention.

EXAMPLE 1

Scavenger receptor protein was purified from
bovine tissue over 2,000-fold by a combination of
maleyl-BSA affinity chromatography (M-BSA),
hydroxyapatite chromatography (HAP), and preparative
NaDodSO$_4$-polyacrylamide gel electrophoresis
(SDS-PAGE) (TABLE 3, Method I). This procedure was
performed at 4°C. Membrane proteins from 500 g of
bovine liver were prepared essentially by the method
of Schneider et al. (Vol. 225, J. Biol. Chem, pp.
11442-11447 (1980)), herein incorporated as
reference. The proteins were resuspended in 500 ml
of 10 mM Tris-HCl, pH 8, 1 mM CaCl$_2$, 0.15 M NaCl and
1 mM PMSF (Buffer A). They were sonicated twice, and
then dissolved by the addition of 55 ml of 20% Triton
X-100 with stirring for 30 min. Insoluble material
was removed by centrifugation (33,000 rpm, 1 hr,
Beckman Type 35 rotor). The supernatant (500 ml) was
applied at 75 ml/hr to an M-BSA-coupled Sepharose 4B
column (Pharmacia, 9.8 x 12 cm, containing about 10
mg of M-BSA/ml of gel) which had been equilibrated
with Buffer A containing 1% Triton X-100. The column
was washed overnight with the same buffer and then
washed with two column volumes of Buffer A containing
40 mM octyglucoside. The receptor protein was eluted

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with Buffer B (1 M NaCl, 20 mM Tris HCl, pH 8, 1 mM
CaCl$_2$, 1 mM PMSF, and 40 mM octyglucoside).

EXAMPLE 2

Filter binding and ligand blotting assays
were performed with minor modification, according to
the methods of Schneider et al. (ibid.) and Daniel et
al. (Vol. 258, J. Biol. Chem. pp. 4606-4611 (1983)),
respectively, herein incorporated as reference.

Ligand binding specificity was also
determined by polynucleic acid affinity
chromatography. M-BSA-purified proteins, having 150
ng of Ac-LDL binding activity in 4 ml of Buffer A
containing 40 mM octyl glucoside, were applied to
polynucleic acid coupled agarose columns (AG-POLY
series, prepacked column, Pharmacia). After washing
with the same buffer, the bound proteins were eluted
with 5 ml of Buffer B.

Cellular scavenger receptor protein activity
was assayed essentially as described by Krieger (Vol.
33., Cell pp. 413-422 (1983)), herein incorporated as
reference, by measuring the degradation of
^{125}I-Ac-LDL (2 mg Ac-LDL protein/ml) in the presence
or absence of inhibitor (M-BSA, 500 mg/ml).

EXAMPLE 3

The fractions obtained as described in
EXAMPLE 1 were tested for their ability to bind
Ac-LDL as described in EXAMPLE 2; those containing
Ac-LDL binding activity (100 ml) w re pooled and

concentrat d (35 ml) using ultrafiltration (Diaflo
m mbrane PM30, Amicon). The sample buffer was
changed to 25 mM potassium phosphate, 40 mM
octyglucoside, 1 mM PMSF, pH 6.8 using PD10 desalting
columns (Pharmacia). The M-BSA affinity purified
fraction (50 ml) was then applied to an Ultrogel-Ha
(LKB) column (2.5 x 13 cm) at a flow rate of 75
ml/hr. The proteins were eluted with a gradient of
phosphate buffer (25 mM to 350 mM) containing 40 mM
octyglucoside.

The 220 kD protein was recovered at
phosphate concentrations between 100 and 200 mM and
was further purified by non-reducing SDS-PAGE on a
3-10% acrylamide gradient gel as described by Laemmli
(Vol. 227, Nature pp. 680-685 (1970)), herein
incorporated as reference). FIG. 1 shows the silver
staining (A, lanes 1, 2, 3) and Ac-LDL ligand
blotting (B, lanes 4, 5, 6) of the purified fractions
on non-reducing gels. A 220 kD protein with Ac-LDL
binding activity was purified. The protein was
electroeluted from the gel in 0.1% SDS, 10 mM
Tris-HCl, pH 8 using an ISCO 1750 electrophoretic
concentrator. TABLE 3 summarizes these results under
Method I.

TABLE 3

	Protein			
Fraction	(mg/frac.)	total (mg)	Spec. Act. (mg/mg)	Pur. Fac. (X)
Method I				
Liver mem.	21,580	107.9	0.005	1
Maleyl-BSA	72	154.8	2.15	430
HAP Chrom.	6	62.8	10.47	2094
Prep. PAGE	0.025*	--	--	--
Method II				
Lung mem.	22,800	136.8	0.006	1
M-BSA Chrom.	45	121.0	2.684	427
Immunoaff.	0.03°	42.0	14030	238,000

* Estimated from chromogeneity on silver staining
of PAGE gels.

** Due to the presence of SDS, the activity
could not be determined.

° Estimated from amino acid composition analysis.

    Partial purification of the 220 kD protein
from other bovine organs or from cells followed
essentially the same procedure as for bovine liver,
except that all steps were performed on a smaller
scale.

    The distribution of the 220 kD protein and
Ac-LDL binding activity in various bovine organs was
examined using immuno- and ligand blotting. There was
close correlation of the intensities of staining and
of the el ctrophoretic mobiliti s (220 kD) of the

antibody binding and ligand binding proteins. Ligand
binding activities and the amounts of 220 kD protein
were high in the liver, lung, spleen and adrenal
gland, which are organs known to take up Ac-LDL
efficiently in vivo. The intestine, which takes up
little Ac-LDL, contained very little 220 kD protein
and little binding activity.

EXAMPLE 4

The scavenger receptor protein was purified
238,000-fold by a combination of M-BSA affinity
chromatography, and IgG-D1 immunoaffinity
chromatography (TABLE 3, Method II). All procedures
were performed at 4°C. 100 ml of Buffer C (0.1% SDS,
0.1% sodium deoxycholate, 1% Nonidet P40, 50 mM
Tris-HCl, pH 8, 150 mM NaCl, and 1 mM PMSF) were
added to M-BSA affinity purified proteins from 500 g
of bovine liver or lung in 100 ml Buffer B. The
sample was applied to Sepharose 4B (Pharmacia)
coupled with IgG-D1 prepared as described below. (4
mg antibody/ml gel, 1 x 2 cm) at a flow rate of 50
ml/hr, and recycled overnight. The column was washed
consecutively with 50 ml of Buffer C, 50 ml of Buffer
D (0.2% Triton X-100, 10 mM Tris-HCl, pH 8), 50 ml of
Buffer D containing 2 M NaCl, and 20 ml of Buffer E
(40 mM octylglucoside containing 10 mM Tris-HCl, pH
8). The bound proteins were then eluted with 20 ml
of Buffer E containing 2 M guanidine thiocyanate.
After elution, the buffer was changed to Buffer A
containing 40 mM octyglucoside using PD10 columns
(Pharmacia). Proteins were stored at -70°C. TABLE 3
summarizes the results under Method II.

EXAMPLE 5

A hybridoma was made by the fusion of mouse
myeloma cells (P3X63-Ag8.653) and spleen cells of a
mouse which had been immunized with receptor protein
protein purified by preparative SDS-PAGE, essentially
as described by Oi et al. (ibid.), herein
incorporated as reference. 15 mg of receptor protein
was used for the initial injection, and 10 mg was
used for the booster. Hybridomas secreting antibody
were identified by an enzyme immunoassay using
96-well plates coated with hydroxyapatite
(HAP)-purified proteins as described by Beisiegel et
al. (Vol. 256, J. Biol. Chem. pp. 11923-11931
(1981)), incorporated herein as reference, and then
by immunoblotting as described by Tsang et al. (Vol.
92, Methods in Enzymology pp. 377-390 (1983)),
incorporated herein as reference. The hybridomas
were subjected to limiting dilution twice and were
cultivated in RPMI 1640 medium containing 0.1% fetal
calf serum and 1% Nutridoma-NS. The monoclonal
antibody IgG-D1, and the control antibody which
recognizes a distinct and apparently unrelated 287 kD
protein, were isolated from the culture media by
Affigel protein A chromatography using a MAPS II
buffer kit (Bio Rad).

EXAMPLE 6

Partially purified receptor protein isolated
as described in EXAMPLE 1 was subjected to SDS-PAGE
and immunoblotting procedures as described by Tsang
et al., (ibid.). The immunoblot shown in FIG. 1(C),
(lane 7) demonstrat s that IgG-D1, raised against the

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unreduced, gel-purified 220 kD bovine protein,
recognizes the bovine 220 kD protein. Reduction
abolishes the binding of IgG-D1 and Ac-LDL ligand
blotting activity.

EXAMPLE 7

The cell type specificity and tissue
distribution of the 220 kD protein and its relation
to the Ac-LDL receptor were also studied using
immunochemical techniques. Immunoprecipitation was
performed essentially as described by Kozarsky et al.
(Vol. 102, J. Cell Biol., pp. 1567-1575 (1986)),
herein incorporated as references using the
monoclonal antibodies obtained as described in
EXAMPLE 3, and using the HAP-purified binding protein
from bovine liver or lung (2-10 mg Ac-LDL binding
activity/mg protein) which had been radioiodinated
using Iodobeads (Pearce). The results of
immunoprecipitation of ^{125}I-labelled specimens with
IgG-D1 or with control antibody T2D2 are shown in
FIG. 5. IgG-D1 specifically precipitated three
^{125}I-labelled bovine species with apparent molecular
weights of 220 kD, 150 kD, and 77 kD (FIG. 5A, lane
1). The immunoprecipitated 220 kD protein was
recovered and shown to retain Ac-LDL binding activity
by ligand blotting (FIG. 5B, lane 5).

EXAMPLE 8

The scavenger receptor proteins precipitated
by IgG-D1 and purified by immunoaffinity
chromatography exhibited apparent molecular weights
of 220 kD, 150 kD, and 77 kD by non-reducing

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SDS-PAGE. After reduction they collapsed into 77 kD
bands (FIG. 5A, lanes 3 and 4). When the 220 kD and
150 kD proteins, obtained by immunoprecipitation or
immunoaffinity chromatography, were individually
recovered from preparative gels and then reduced, the
apparent mass of each was also converted to 77 kD,
indicating that the 220 kD and 150 kD proteins were
trimers and timers of 77 kD subunits.

The carbohydrate characteristics of the
receptor protein were determined by subjecting the
77,000 dalton subunit to immunoprecipitation (as
described in EXAMPLE 7) followed by digestion with
sialidase (Boehringer Mannheim, Indianapolis,
Indiana) or endoglycosidase F (GENZYME, Boston, MA)
as described by Elder et al. (Vol. 79, Proc. Natl.
Acad. Sci. (USA), pp. 4540-4544 (1982)) herein
incorporated as reference. The resulting protein was
then subjected to SDS-PAGE under reducing conditions
(FIG. 6).   After treatment, Its apparent molecular
weight was reduced by approximately 5 kD after
sialidase treatment, or 13 kD after endoglycosidase
treatment, demonstrating the presence of
asparagine-linked carbohydrate chains and sialic
acids.

The immunoaffinity-purified activity also
exhibited apparent molecular weights of 220 kD, 150
kD, and 77 kD by non-reducing SDS-PAGE (FIG. 5C, lane
7).

EXAMPLE 9

Immunohistochemisty was performed on bovine
liver samples as follows. Tissue sections (6 mm)
were cut on a cryostat at -20°C, placed on glass
slides, air dried for 1 hour, and fixed in cold 95%
methanol. The sections were incubated with
monoclonal antibody IgG-D1 (6 mg/ml) or control mouse
IgG. The binding of IgG was visualized using
peroxidase-conjugated sheep anti-mouse IgG Fab by
3,3'-diaminobenzidine tetrahydrochloride staining as
described by Adamson et al., (Vol. 130, J. Immunol.
pp. 203-207 (1983)), herein incorporated as
reference. Sections were counterstained with methyl
green (Sigma).

FIG. 7 shows the immunohistochemical
analysis of frozen liver sections using the
anti-bovine 220 kD protein monoclonal antibody
IgG-D1. The distribution of the 220 kD binding
protein matches that previously described for
scavenger receptor protein activity: specific
sinusoidal (arrows), but not hepatocyte (H),
immunostaining (panels B and C); and very little
staining in the area immediately surrounding the
central vein (CV). P indicates portal area.

EXAMPLE 10

Cell cultures of macrophages were prepared
to test their ability to take up acetyl-LDL. Bovine
alveolar macrophages were collected from fresh lung
by bronchial lavage with phosphate buffered saline
(PBS, 1 liter/lung). They were cultivated with RPMI

1640 medium containing 10% fetal calf s rum for 6 hrs
at 37°C in a CO_2 incubator, and washed with PBS 6
times to remove non-adherent cells. The uptake of
fluorescent Ac-LDL labelled with 1,1'-dioctadecyl-
3,3,3'3'-tetramethyl-indocarbocyanine (DiI) was
performed as described by Kingsley et al. (Vol. 81,
Proc. Natl. Acad. Sci. (USA) pp. 5454-5458 (1984)),
herein incorporated as reference.

For immunoblotting, cells from two 150 mm
dishes were incubated with trypsin (0.05%) or PBS for
5 min at 37°C, and harvested by scraping. Floating
cells were recovered by centrifugation and were mixed
with the scraped cells. The cells were then
resuspended in 10 ml of ice cold Buffer A. The 220
kD protein, partially purified from the membrane
using M-BSA affinity chromatography, was subjected to
immunoblotting as described in EXAMPLE 6. The
viability of cells, determined by either trypan blue
staining (floating cells) or restoration of
DiI-Ac-LDL uptake after overnight cultivation
(adherent cells), was not significantly affected by
the mild trypsin digestion.

Coincident expression of Ac-LDL receptor
activity (fluorescent DiI-Ac-LDL accumulation, FIG. 8
panel A) and the 220 kD protein (immunoblotting with
IgG-D1, FIG. 8, panel B, lane A) was also detected in
cultured bovine aveolar macrophages. Fluorescently
labelled Ac-LDL uptake was blocked by the presence of
excess unlabelled Ac-LDL (panel A). Immunodetection
of the 220 kD protein was prevented by pretreating
the macrophages with low, sublethal levels of trypsin

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(0.05%) for 5 min. at 37°C (FIG. 8, panel B, lan
B). The trypsin sensitivity of this protein in
intact cells suggests that some of the 220 kD protein
is at least partially exposed at the cell surface.

EXAMPLE 11

The human monocytic leukemia cell line THP-1
was cultivated in RPMI 1640 medium containing 10%
fetal calf serum. THP-1 cells were induced to
differentiate into macrophage-like cells by
cultivation for 3 days in the presence of 200 nM
phorbol 12-myristate 13-acetate (PMA). The
maleyl-BSA affinity purified membrane proteins from
10^8 cells treated with or without PMA were subjected
to Ac-LDL ligand blotting and filter binding assays
as described in EXAMPLE 1.

Coincident and coordinately regulated
expression of acetyl-LDL receptor activity and a 220
kD binding protein were observed in this human cell
line. FIG. 9 shows that the phorbol ester induction
of receptor activity (^{125}I-Ac-LDL degradation assay)
is accompanied by a substantial increase in the
^{125}I-Ac-LDL binding activity of cell membrane
proteins (filter binding assay), and by a dramatic
increase in the amount of activity of a 220 kD
scavenger receptor protein (ligand blot assay). The
immunoaffinity-purified receptor protein bound
^{125}I-Ac-LDL with an apparent dissociation constant of
0.5 mg/ml (0.8 nM) (FIG. 10A). The maximum binding
of ^{125}I-Ac-LDL was about 1.4 mg/mg.

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Comp tition of Ac-LDL binding was measured
by preincubating immunoaffinity purified receptor
protein/phospholipid complexes in the various
concentrations of analyte competitors for 30 min.
prior to the addition of ^{125}I-Ac-LDL (2 mg
protein/ml) for 1 hour at 4°C. (Relatively
successful analyte competitors were LDL, maleyl-BSA,
fucoidan, polyvinylsulfate, and the purine
polynucleotides poly[I], poly[I-C], and poly[G] (FIG.
10B).

The purified receptor can also bind Ox-LDL.
TABLE 4 demonstrates this activity. The OX-LDL was
prepared using the method of Steinbrecher et al.
(Vol. 81, Proc. Natl. Acad. Sci (USA) pp. 3883-3887
(1984)), herein incorporated as reference. The
specific activity was determined by subtracting the
binding activity in the presence of competitor (200
ug/ml Ac-LDL) from the activity in the absence of
competitor by the liposome reconstitution assay using
^{125}I-Ox-LDL. These result indicates that the
scavenger receptor protein may also play an important
role in the metabolism of Ox-LDL.

TABLE 4

Oxidized-LDL (mg/ml)	Specific Binding of ^{125}I-Ox-LDL (µg Ox-LDL/mg scavenger receptor)
0.5	59
1.0	89
2.0	135
5.0	302
10.0	417

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EXAMPLE 12

The scavenger receptor protein was further purified using RP-300 (Brownlee Laboratory, Emeryville, CA) reverse phase HPLC. The receptor was solubilized in 70% formic acid. It was then cleaved by cyanogen bromide (CNBr) (25 mg/100 ml solution) at room temperature, overnight, resulting in the formation of a number of cleavage fragments. The fragments were chromotographically separated on the RP-300 column.

One of the fragments was isolated and subjected to automated amino acid analysis using an Applied Biosystems amino acid sequencer (Foster City, CA). The following amino acid sequence was thereby obtained:

Arg-Ile-Gln-Tyr-Leu-Ser-Asp-Asn-Glu-Ala-

Asn-Leu-Leu-Asp-Ala-Lys-Asn-Phe-Gln.

A second sequence (below) was obtained from a similar CNBr digestion using the gel elctrophoresis/ immunoblotting method of Matsudaira (J. Biol. Chem. (1987) 262:10035-10038):

Lys-Leu-Leu-Asn-X-Ile-X-Asn-Asp-Leu-Arg-Leu-X-Asp-Trp

where "X" indicates that the amino acid residue at that position is uncertain. (See FIGs. 3A-C for the relative location of these residues (underscored dots) in the amino acid sequence of clone 3.)

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EXAMPLE 13

Methodology for the development and
identification of the clones, unless otherwise noted,
were standard procedures such as those described in
Maniatis et al. (Molecular Cloning, A Laboratory
Model (1982) Cold Spring Harbor Laboratory) and Davis
et al. (Basic Methods In Molecular Biology (1988)
Elsevier Scientific Publishing Co., Inc., NY).

A. Preparation of cDNA Libraries

Poly(A)$^+$ mRNA was isolated by acid guanidium
thiocyanate/phenol/chloroform extraction as described
by Chomczynski (Anal. Biochem. (1987) 162:156) from
bovine lung, and was used to construct a random
primed cDNA library. 6 µg of bovine lung macrophage
mRNA and 5 µg of random hexanucleotides were used to
synthesize double-stranded cDNA following the general
procedure of Gubler and Hoffman (Gene (1983)
25:263). The cDNA so synthesized was ligated to Eco
R1 adaptors (Pharmacia), and was then
size-fractionated on a 5-20% potassium acetate
gradient by centrifugation for 3 hr in an SW60 rotor
(Beckman Instruments) at 50,000 rpm. The cDNA
fractionating at molecular masses of approximately
greater than or equal to 800 base pairs (by agarose
electrophoresis) was then ligated into Eco R1
digested λzap (Stratagene), packaged, and
subsequently used to infect XL-1 Blue cells
(Stratagene). 1.5 x 10^6 primary clones were obtained
and amplified.

A human cDNA library was pr pared from the
human monocytic leukemia cell line THP-1 using
equivalent methodologies.

B.   Library Screening and Isolation of cDNA Clones

The bone and human libraries were screened
with two pools of 41mer oligonucleotide probes that
included 5-fluorodeoxyuridine (F) (Habener et al.
(1988) Proc. Natl. Acad. Sci. (USA) 85:1735-1739)
according to the amino-acid sequence:

RIQULNSDNEANLLDA.

These pools were divided according to the codon usage
at serine. Pool I contained:

5'-ATFCAGTAFFT(F/G)TC(F/G)GAFAAFGA
   GGCF/G)AAFFT(F/G)FT(F/G)GAGAFGC-3'.

Pool II contained AGF at the underlined (TC(F/G))
nucleotides shown above for pool I. For each pool, 5
x 10^5 plaques were screened using ^{32}P end-labelled
probes by hybridization at 37°C and washing at 50°C
in 6 x SSC (Maniatis et al. ibid. pp. 447) with 0.1%
SDS. Putative positive clones were purified and in
vivo excised into picoblue (pBluscript)-derived
plasmids (Short, ibid.). The inserts were screened
by Southern blot hybridization (Southern (1975) J.
Mol. Biol. 98:503) using 4 pools of the 17mer
oligonucleotide:

A(A/G)(A/G)TTNGC(C/T)TC(A/G)TT(A/G)TC,

each containing a different nucleotide at the underlined (N) position. Pool III contained:

AT(F/A)CA(G/A)TAFFTTTCTGAFAAFGA(G/A)GC

and pool IV contained AGF at the underlined (TC) position of pool III.

Five positive bovine clones were selected and sequenced according to the Sanger method (Sanger et al. (1977) Proc. Natl. Acad. Sci. (USA) 74:5463). These clones have a nucleotide sequence corresponding to the peptide sequence. FIGs. 3A-C show the nucleic acid sequence and corresponding deduced amino acid sequence of clone 3, while FIGs. 4A-D show a composite nucleic acid sequence and corresponding deduced DNA sequence of clones 7, 12, and 13. The human cDNA library was also screened with probes derived from the bovine cDNA using equivalent methodology. FIGs. 14A-B show the nucleic acid sequence and correspondingly deduced amino acid sequence derived from human clones.

C.   Sequence Analysis

The sequencing of the cDNA bovine clones indicates that the cDNAs isolated are derived from two different mRNAs. Both of these mRNAs encode identical 347 amino acid N-terminal domains [$Met^1 \rightarrow ARG^{347}$]. They differ in this region by one nucleic acid which does not result in an amino acid change. The two mRNAs begin to differ at amino acid position 348. The sequence of clone 3 (FIGs. 3A-C) continues for only 2 additional amino acids before a

stop codon is seen. (Arg-Pro-Gly STOP). In
contrast, the s quence of clone 7 (FIGs. 4A-D)
continues for an additional 106 amino acids.

Clone 3 contains 35 noncoding bases at its
5'end, 1047 bases of coding region, and 965 noncoding
bases at its at the 3'end (FIGs. 3A-C). In coding
region (* denotes the start of the coding region),
amino acid residues 1-50 represent the cytoplasmic
domain (Cy), residues 51-76 represent the proposed
transmembrane domain (TM), residues 77-271 represent
the proposed Asn-linked sugar domain, residues
272-343 represent the proposed collagen domain (Col),
and residues 344-349 represent the proposed
C-terminal domain (C-term). In FIGs. 3A-C, the
residues of the TM domain are underscored (first
underscored region); the Asn-linked sugar sites are
overscored; the Col domain is underscored (second
underscored region); M* is the initiator Met; and the
first 19 amino acids making up the peptide used to
generate the anti-peptide antibody are underscored
with dots (with the exception of Ser^{138}, which was
later determined to be Lys).

Clone 7 (FIGs. 4A-D) has no 5' noncoding
bases, 1359 bases of coding region, and 211 noncoding
bases at its 3'end.

D. Expression of cDNA Clones

A clone 3 expression vector pXAxLDLR3 was
prepared by excising the clone 3 sequence from the
pBluescript product (i.e., the in vivo excision
product of the λzap vactor) using BAM Hl and Xhol.

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The r sulting DNA fragment was th n ligated into the pcDNA1 plasmid (Invitrogen) which had been previously cut with the same restriction enzymes.

On day 0, monkey COS M6 cells (derived from COS 7 cells available from the American Type Culture Collection, Rockville, MD, and a gift from D. Hausman, Dept. of Biology, MIT) were set at 1.5×10^6 cells/100 mm dish in the standard cell culture medium DMEM (Gibco) containing 10% fetal bovine serum, penicillin, and streptomycin. On day 1, the cells were tranfected following standard procedures (Seed et al. (1987) Proc. Natl. Acad. Sci. (USA) 84:3365-3369). In brief, a transfection cocktail was added to each dish already containing 4 ml DMEM containing 10% Nuserum (Collaborative Research). The cocktail consisted of 10 µl (40 mg/ml) DEAE Dextran solution, (Pharmacia), 40 µl of 10 mM chloroquine (Sigma), and 4 µg clone 3 expression vectorp λAcLDLR3. The final concentrations in the transfection medium were 10% NuSerum, 100 µg/ml DEAE Dextran, 100 µM chloroquine, and 4 µg/ml vector DNA. After incubation for 4 hr at 37°C, the tranfection medium was removed and the cells treated for 2 min with Hepes buffered saline (HBS: 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM dextrose, 21 mM Hepes, pH 7.1) containing 10% dimethylsulfoxide (DMSO). The HBS-DMSO solution was removed and the cells refed with standard culture medium (DMEM containing 10% fetal bovine serum and the antibiotics). On day 2, the transfected cells were harvested by trypsin treatment. They were reset into 6-well dishes at a concentration of 1×10^6 per well for assays

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performed on day 4, or at a concentration of 0.8 x
10^6 per well for assays performed on day 5.

E.   Receptor Activity Assay

        The ability of the transfected monolayers to
degrade 5 µg protein/ml of ^{125}I-Ac-LDL in the absence
(triplicate determinations) or presence (single
determinations) of different concentrations of
various unlabeled competetitors was measured using
the standard assay procedures of Krieger (ibid.).

        The results are shown in FIG. 11. In
control cells tranfected with pcDNA which did not
contain clone 3, the amount of Ac-LDL degraded was
only 5% of that degraded by the pAcLDL3-transfected
cells. This experiment demonstrates that high
affinity Ac-LDL receptor activity is expressed in
cells transfected with an expression vector including
clone 3 (pXAcLDLR3).

        In another experiment, cells were prepared
and assays were performed as described above except
that 400 µg/ml competitor was used. The inhibitors
included poly [I], poly [C], LDL, Ac-LDL, maleylated
bovine serum albumin (M-BSA), Ox-LDL, BSA, and
polyvinyl sulfate (PVS). The results are shown in
FIG. 12. This experiment also indicates that clone 3
can express high affinity Acetyl-LDL receptor
activity in cells transfected with the clone
3-containing vector pXAcLDLR3.

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F.   Tissue and Cell Type-Specific Expression of
Ac-LDL Receptor mRNA

        The ability of Ac-LDL encoding-clones to be
expressed in different cell and tissue types was
investigated to determine if the pattern of their
expression mirrored that of the expression of the
native receptor. Standard Northern (RNA) blot
hybridization methods were followed using the
collagen domain-encoding fragment of clone 3 as a
probe. THP-1 cells were cultivated with or without
200 nM phorbol 12-myristate 13-acetate (PMA) for 3
days to induce them to differentiate into
macrophage-like cells (Kodama et al. (1988) Proc.
Natl. Acad. Sci. (USA) 85:9238 (Dec.)). Bovine
alveolar macrophages were prepared as previously
described (Kodama, ibid.). Poly(A)$^+$ RNA (3 µg) from
these cells and from various bovine organs was
isolated and blotted on a cellulose nitrate
membrane. The Xba 1/Sph 1 fragment of a subclone
pJAL 5, which includes the sequence encoding the
collagen-like domain of clone 3, was 32p
radiolabelled, hybridized at 42°C in the presence of
40% formamide, and washed with 2 x SSC containing
0.1% SDS at 55°C.

        The results are shown in FIG. 13(A). RNA
sequences encoding the Col domain are detected in
THP-1 PMA(+) (differentiated) macrophages as well as
in alveolar macrophages and lung. This pattern of
RNA expression is the same as the pattern of in vivo
receptor expression.

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To simultaneously determine the amount of
receptor activity in the same tissue specimens,
receptor protein was purified from these bovine
tissues (5 g per organ). The tissue was homogenized,
maleyl-BSA-affinity purified, and subjected to
immunoblotting using monoclonal anti-bovine receptor
antibody IgG-Dl as described previously (Kodama,
ibid.).

The results of this immunoblotting analysis
are shown in FIG. 12(B). Receptor was detected in
lung, but not in brain or muscle, as expected from
the Northern blot results described above.

It can be seen from the foregoing
description and examples that a receptor protein
having a high affinity for Ac-LDL can be isolated and
purified to near homogeniety from various appropriate
mammalian tissues. Monoclonal antibodies can be made
to this receptor protein which can aid in its
identification and further purification, and which
are useful in methods of detecting cellular
structures that contain the receptor protein such as
atherosclerotic plaques. In addition, DNA clones can
be prepared which encode at least a portion of the
scavenger recepter protein, and that these clones
have the same pattern of differential expression in
various tissue types as does the native receptor.
Furthermore, the expressed protein has scavenger
recepter protein-like activity.

The invention may be embodied in other
specific forms without departing from the spirit or

ssential characteristics thereof. The present
embodiments are therefore to be considered in all
respects as illustrative and not restrictive, the
scope of the invention being indicated by the
appended claims rather than by the foregoing
description, and all changes which come within the
meaning and range of equivalency of the claims are
therefore intended to be embraced therein.

        We claim:

1.   A substantially pur r ceptor protein
capable of binding ac tylated low density
lipoprotein, said protein characterized by:
            having an apparent molecular weight on
            SDS-polyacrylamide gels of about 220,000
            daltons; and
            having an affinity for acetylated low
            density lipoprotein.

2.   An active fragment, analog, or derivative of
said receptor protein of claim 1 capable of binding
acetylated low density lipoprotein.

3.   The receptor protein of claim 1 further
characterized as being a glycoprotein.

4.   The receptor protein of claim 3 further
comprising an asparagine-linked carbohydrate chain.

5.   The receptor protein of claim 1 further
characterized as comprising a trimer of subunits,
each of said subunits having an apparent molecular
weight on polyacrylamide gels of about 77,000 daltons.

6.   The receptor protein of claim 5 wherein each
of said subunits comprises an asparagine-linked
carbohydrate chain.

7.   The receptor protein of claim 1 further
characterized by having an affinity for acetylated
low density lipoprotein of about 0.5 micrograms per
milliliter.

8.   The r ceptor protein of claim 1 further
characterized by the ability to bind a negatively
charged macromolecule selected from the group
consisting of polyvinylsulfate, maleyl-BSA, fucoidan,
and purine polynucleotides, poly[I-C], poly[I] and
poly[G].

9.   A binding reagent characterized by having
the ability to bind the scavenger receptor protein,
        said receptor protein being characterized by
having an apparent molecular weight on polyacrylamide
gels of about 220,000 daltons, and having an affinity
for acetylated low density lipoprotein.

10.   The binding reagent of claim 9 where said
reagent is a biologically active molecule selected
from the group consisting of antibodies, fragments,
analogs, and derivatives thereof retaining the
ability to react with said scavenger receptor protein.

11.   The binding reagent of claim 9 further
comprising monoclonal antibodies.

12.   The binding reagent of claim 9 further
comprising a labelling means.

13.   The antibody of claim 12 wherein said
labelling means comprises a radioisotope.

14.   A device for assay or purification purposes,
said device comprising:

            a support; and

            a plurality of binding reagents bound
      to said support, each of said binding
      reagents being characterized by having the
      ability to bind acetylated low density
      lipoprotein.

15.   The device of claim 14 wherein each of said
reagents is a biologically active molecule selected
from the group consisting of antibodies, fragments,
analogs, and derivatives thereof retaining the
ability to react with said scavenger receptor protein.

16.   The device of claim 15 wherein said reagents
comprise monoclonal antibodies.

17.   A device for assay or purification purposes,
said device comprising:

            a support; and

            a plurality of receptor proteins bound
      to said support, each of said receptor
      proteins being characterized by having an
      apparent molecular weight on polyacrylamide
      gels of about 220,000 daltons, and the
      ability to bind acetylated low density
      lipoprotein.

18.   The device of claim 17 wherein said receptor
proteins are selected from the group consisting of
active fragments, analogs, and derivatives of said
receptor protein capable of binding oxidized low
density lip protein.

19.   A method of detecting an atherosclerotic
plaque in the vascular system of a subject, said
method comprising the steps of:

        coupling a binding agent to a labelling
        means to form a conjugate, said binding
        agent having a specific affinity for a
        scavenger receptor protein associated with a
        macrophage, said macrophage being associated
        with said plaque;

        administering said conjugate to the
        vascular system of said subject;

        allowing said binding agent of said
        conjugate to bind to said scavenger receptor
        protein; and

        detecting the presence and location of
        said labelling means of said conjugate in
        the vascular system of said subject.

20.   The method of claim 19 wherein said coupling
step includes coupling said labelling means to a
binding reagent selected from the group consisting of
antibodies, fragments, analogs, and derivatives
thereof, each of said group retaining the ability to
react with said scavenger receptor protein.

21.   The method of claim 20 wherein said coupling
step further comprises coupling said labelling means
to a monoclonal antibody.

22.   The method of claim 19 wherein said coupling
step further comprises coupling said binding reagent
to a radioisotope, and said detecting step comprises
detecting said radioisotope by extracorporeal
radioactivity-d t cting means.

23.   A method of selectively delivering a
therapeutic agent to an atherosclerotic plaque in the
vascular system of a subject, said method comprising
the steps of:

    coupling a therapeutic agent to a
    binding agent to form a conjugate, said
    binding agent having a specific affinity for
    a scavenger receptor protein on a
    plaque-forming component associated with
    said plaque; and

    administering said conjugate to the
    vascular system of said subject, said
    conjugate binding to said receptor protein,
    thereby delivering said therapeutic agent to
    said plaque-forming component and said
    plaque.

24.   The method of claim 23 wherein said coupling
step further comprises coupling said therapeutic
agent to a binding agent, said binding agent
including oxidized low density lipoprotein.

25.   The method of claim 23 wherein said coupling
step further comprises coupling said therapeutic
agent to a binding agent, said binding agent
including acetylated low density lipoprotein.

26.   The method of claim 23 wherein said coupling
step further comprises coupling said therapeutic
agent to a binding agent, said binding agent
including an antibody having a specific affinity for
said scavenger receptor protein.

27.   The meth d of claim 26 wh rein said coupling
step further comprises coupling said therapeutic
agent to a binding agent, said binding agent
including a monoclonal antibody.

28.   The method of claim 26 wherein said coupling
step further comprises coupling said therapeutic
agent to a binding agent selected from the group
consisting of antibodies, fragments, analogs, and
derivatives thereof, each of said group retaining the
ability to react with said scavenger receptor protein.

29.   A method of treating vascular disease
comprising the steps of:
          administering to the vascular system of
     a subject a therapeutic agent, said agent
     including an antagonist of the scavenger
     receptor protein; and
          allowing said antagonist to bind to
     said receptor protein on a plaque forming
     component so as to inhibit the contribution
     of such plaque forming component to vascular
     disease.

30.   The method of claim 29 wherein said
administrating step comprises administering a binding
molecule to the vascular system, said binding
molecule having a specific affinity for said receptor
protein.

31.   The method of claim 30 wherein said
administrating st p further comprises administering a
binding molecule to the vascular system, said binding
molecule including an antibody having a specific
affinity for said receptor protein.

32.   The method of claim 31 wherein said
administrating step comprises administering an
antibody to the vascular system, said antibody
including a monoclonal antibody having a specific
affinity for said receptor protein.

33.   A polypeptide comprising an amino acid
sequence sufficiently duplicative of the amino acid
sequence of at least one subunit of the scavenger
receptor protein such that the polypeptide, when
combined with one or more additional polypeptides or
native subunits of said scavenger receptor protein,
binds chemically modified forms of LDL.

34.   A DNA sequence comprising a sequence
encoding at least a portion of the protein of claim 1
or 2.

35.   A DNA sequence comprising a sequence
encoding at least a portion of the polypeptide
subunit of claim 33.
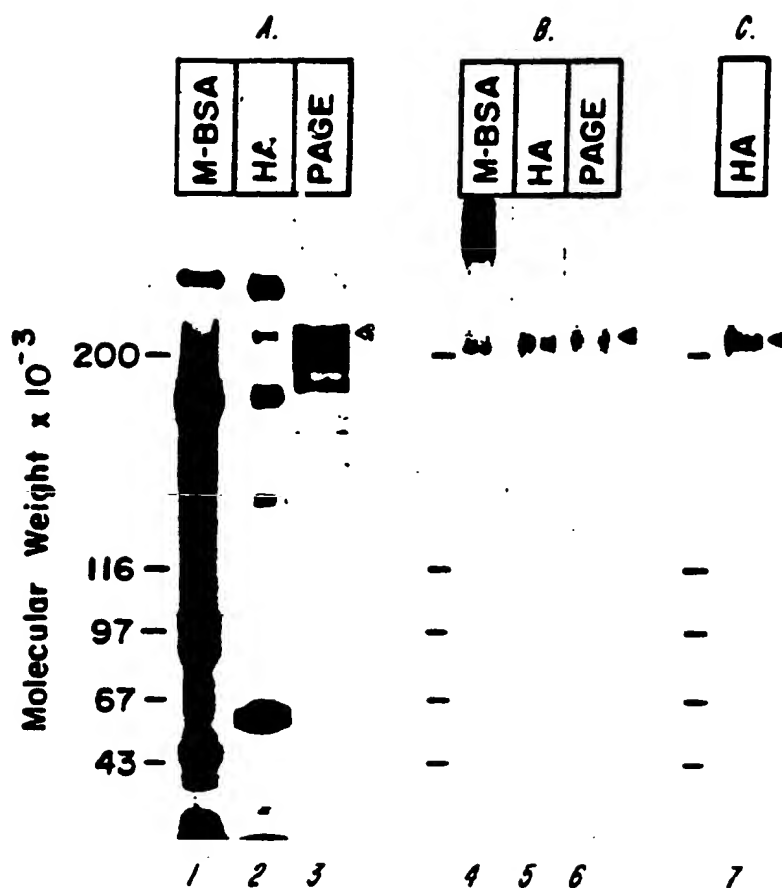
FIG. 1
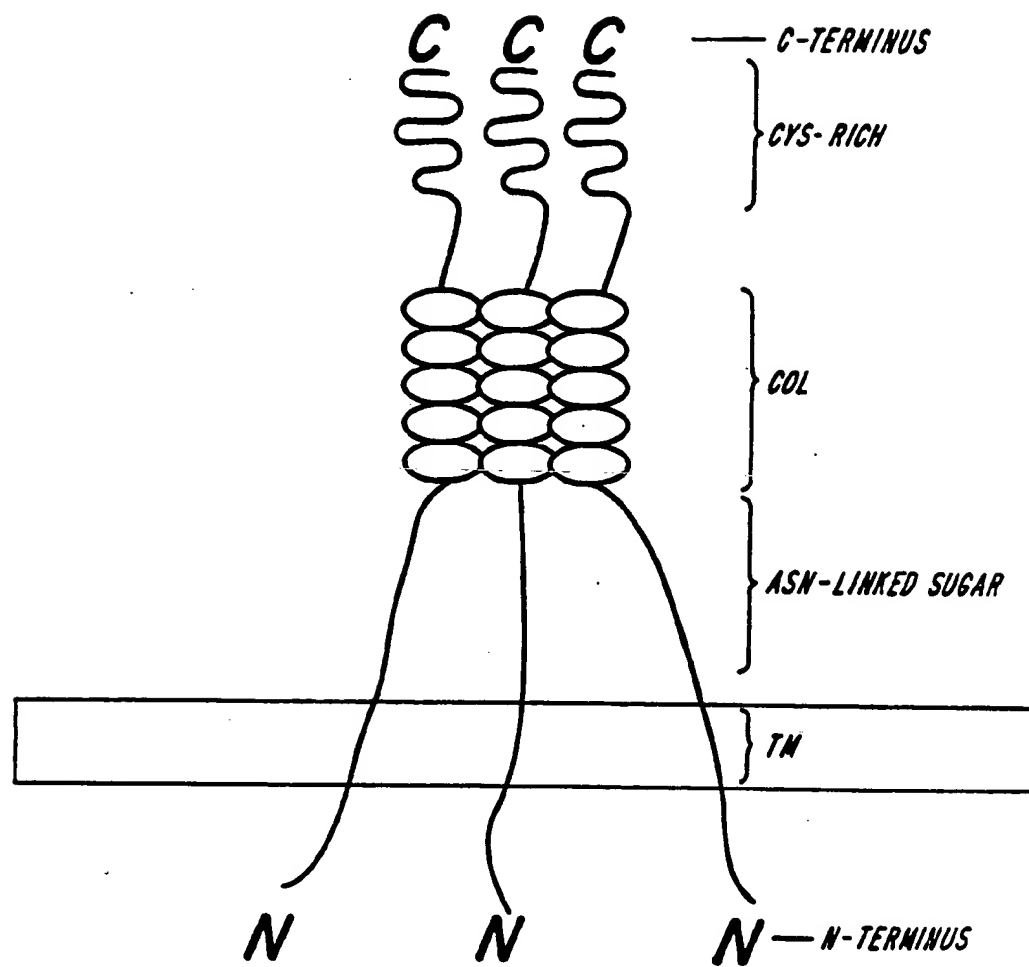
FIG. 2

```
1                                                      50
TAAATCGGTGCTGCCGTCTTTAGGACATATGAAGTATGGCACAGTGGGAT
                                  1              5
                                  M*  A   Q   W   D

51                                                     100
GACTTTCCTGATCAGCAAGAGGACACTGACAGCTGTACAGAGTCTGTGAA
6                                                      22
D   F   P   D   Q   Q   E   D   T   D   S   C   T   E   S   V   K

101                                                    150
GTTCGATGCTCGCTCAGTGACAGCTTTGCTTCCTCCCCATCCTAAAAATG
23                                                     39
 F   D   A   R   S   V   T   A   L   L   P   P   H   P   K   N   G

151                                                    200
GCCCAACTCTTCAAGAGAGGATGAAGTCTTATAAAACTGCACTGATCACC
 40                                                    55
  P   T   L   Q   E   R   M   K   S   Y   K   T   A   L   I   T

201                                                    250
CTTTATCTCATTGTGTTTGTAGTTCTCGTGCCCATCATTGGCATAGTGGC
56                                                     72
L   Y   L   I   V   F   V   V   L   V   P   I   I   G   I   V   A

251                                                    300
AGCTCAGCTCCTGAAATGGGAAACGAAGAATTGCACGGTTGGCTCAGTTA
73                                                     89
 A   Q   L   L   K   W   E   T   K   N   C   T   V   G   S   V   N

301                                                    350
ATGCAGATATATCTCCAAGTCCGGAAGGCAAAGGAAATGGCAGTGAAGAT
 90                                                    105
  A   D   I   S   P   S   P   E   G   K   G   N   G   S   E   D

351                                                    400
GAAATGAGATTTCGAGAAGCTGTGATGGAACGCATGAGCAACATGGAAAG
106                                                    122
E   M   R   F   R   E   A   V   M   E   R   M   S   N   M   E   S

401                                                    450
CAGAATCCAGTATCTTTCAGATAATGAAGCCAATCTCCTAGATGCTAAGA
123                                                    139
 R   I   Q   Y   L   S   D   N   E   A   N   L   L   D   A   K   N
 .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
```

FIG. 3A

```
451                                                  500
ATTTCCAAAATTTCAGCATAACAACTGATCAAAGATTTAATGATGTTCTT
140                                             155
  F  Q  N  F  S  I  T  T  D  Q  R  F  N  D  V  L
     •  •

501                                                  550
TTCCAGCTAAATTCCTTACTTTCCTCCATCCAGGAACATGAGAATATCAT
156                                             172
  F  Q  L  N  S  L  L  S  S  I  Q  E  H  E  N  I  I

551                                                  600
AGGGGATATCTCCAAGTCATTAGTAGGTCTGAACACCACAGTACTTGATT
173                                             189
  G  D  I  S  K  S  L  V  G  L  N  T  T  V  L  D  L

601                                                  650
TGCAGTTCAGTATTGAAACACTGAATGGCAGAGTCCAAGAGAATGCATTT
190                                             205
  Q  F  S  I  E  T  L  N  G  R  U  Q  E  N  A  F

651                                                  700
AAACAACAAGAGGAGATGCGTAAATTAGAGGAGCGTATATACAATGCATC
206                                             222
  K  Q  Q  E  E  M  R  K  L  E  E  R  I  Y  N  A  S

701                                                  750
AGCAGAAATTAAGTCTCTAGATGAAAAACAAGTATATTTGGAACAGGAAA
223                                             239
  A  E  I  K  S  L  D  E  K  Q  U  Y  L  E  Q  E  I

751                                                  800
TAAAAGGGGAAATGAAACTGTTGAATAATATCACTAATGATCTGAGGCTG
240                                             255
  K  G  E  M  K  L  L  N  N  I  T  N  D  L  R  L
        •  •        •        •     •  •  •     •

801                                                  850
AAGGATTGGGAACATTCTCAGACATTGAAAAATATCACTTTACTCCAAGG
256                                             272
  K  D  W  E  H  S  Q  T  L  K  N  I  T  L  L  Q  G
     •  •

851                                                  900
TCCTCCTGGACCTCCAGGTGAAAAAGGAGATAGAGGCCCTCCTGGACAAA
273                                             289
  P  P  G  P  P  G  E  K  G  D  R  G  P  P  G  Q  N
```

FIG. 3B

```
901                                                    950
ATGGTATACCAGGCTTTCCAGGTCTAATAGGTACTCCAGGTCTTAAAGGT
290                                                    305
  G   I   P   G   F   P   G   L   I   G   T   P   G   L   K   G


951                                                    1000
GATCGGGGGATCTCTGGTTTACCTGGAGTTCGAGGATTCCCAGGACCAAT
306                                                    322
 D   R   G   I   S   G   L   P   G   V   R   G   F   P   G   P   M


1001                                                   1050
GGGGAAGACCGGGAAGCCAGGACTTAATGGACAAAAAGGCCAGAAGGGAG
323                                                    339
  G   K   T   G   K   P   G   L   N   G   Q   K   G   Q   K   G   E


1051                                                   1100
AAAAAGGGAGTGGAAGCATGCAAAGACCAGGATAACTCCCTGATCGTATT
340                      349
  K   G   S   G   S   M   Q   R   P   G   *


1101                                                   1150
AGGCAGGCCCCTTTGAAGATCAGGTGGGTTGGGCGGGACACCCCGTGCTA
1151                                                   1200
CCATCTCATTAAAAGGCCCTTCATCTCTGGACAAGTCATCAGCCACATCT
1201                                                   1250
GACTCCAGGATTCCCTTGTGGCTCCTCCAAACCGACCTTGGTTCCCACGT
1251                                                   1300
GGTGTCAGCTACCTGTCCGCTCTCCAGCACCTCTCCATGACCACTGTCCC
1301                                                   1350
TGAGCTGGGATGCCTCCATGACTCACTCATCTAGTGTCTCCCGAATCATT
1351                                                   1400
GGCTCCCAGGAGAAATTTCATGGTAACAGATTATATGTGAGTTCTTTACC
1401                                                   1450
CTCAAACATATCAGTCTCAGCCAGAGATAGTGATGCCATACAGCTAATAA
1451                                                   1500
GATAGCTGTTGTCTCTGGCCTTCCAGAACTAATTGTCCCTGAAAAACATA
1501                                                   1550
GCCATGGACACAAGCAGTTATGCATTAGAGCAGGGCTAGATGGGGGAAGG
1551                                                   1600
AACTGGGCTCCGTGGGTGTTCAGAGGGGATCAAGAGTCTTCAATTCCTTA
1601                                                   1650
GAATTTTAAAAAGGTAAATTCAAGTTTTGTCATGAAGTCAAGTGGAGCTG
1651                                                   1700
GGGCAGTTCCTTCAAAATTTTTTAGCCAAAAAAAAAAAAAATCAAAATTT
1701                                                   1750
GTTGCCTTAAATTACCAGGCTCTTTCAAGCAAGAATATTTTGACCATAAA
1751                                                   1800
AAAATAATGCTGAAACTATTTTATTCTATAAAGCTCTTTTCACCAGCCCT
1801
CGTGCCG
```

FIG. 3C

```
126                                                    170
AGTATGGCACAGTGGGATGACTTTCCTGATCAGCAAGAGGACACTGACAG
   1                                                16
   M* A   Q   W   D   D   F   P   D   Q   Q   E   D   T   D   S

173                                                    220
CTGTACAGAGTCTGTGAAGTTCGATGCTCGCTCAGTGACAGCTTTGCTTC
17                                                    33
   C   T   E   S   V   K   F   D   A   R   S   V   T   A   L   L   P

223                                                    270
CTCCCCATCCTAAAAATGGCCCAACTCTTCAAGAGAGGATGAAGTCTTAT
   34                                                  49
    P   H   P   K   N   G   P   T   L   Q   E   R   M   K   S   Y

273                                                    320
AAAACTGCACTGATCACCCTTTATCTCATTGTGTTTGTAGTTCTCGTGCC
50                                                    66
   K   T   A   L   I   T   L   Y   L   I   V   F   V   V   L   V   P

323                                                    370
CATCATTGGCATAGTGGCAGCTCAGCTCCTGAAATGGGAAACGAAGAATT
67                                                    83
    I   I   G   I   V   A   A   Q   L   L   K   W   E   T   K   N   C

373                                                    420
GCACGGTTGGCTCAGTTAATGCAGATATATCTCCAAGTCCGGAAGGCAAA
84                                                    99
    T   V   G   S   V   N   A   D   I   S   P   S   P   E   G   K

423                                                    470
GGAAATGGCAGTGAAGATGAAATGAGATTTCGAGAAGCTGTGATGGAACG
100                                                   116
   G   N   G   S   E   D   E   M   R   F   R   E   A   V   M   E   R

473                                                    520
CATGAGCAACATGGAAAGCAGAATCCAGTATCTTTCAGATAATGAAGCCA
117                                                   133
   M   S   N   M   E   S   R   I   Q   Y   L   S   D   N   E   A   N

523                                                    570
ATCTCCTAGATGCTAAGAATTTCCAAAATTTCAGCATAACAACTGATCAA
134                                                   149
    L   L   D   A   K   N   F   Q   N   F   S   I   T   T   D   Q
```

FIG. 4A

```
573                                                      620
AGATTTAATGATGTTCTTTTCCAGCTAAATTCCTTACTTTCCTCCATCCA
150                                                      166
R   F   N   D   V   T   F   Q   T   N   S   L   L   S   S   I   Q

623                                                      670
GGAACATGAGAATATCATAGGGGATATCTCCAAGTCATTAGTAGGTCTGA
167                                                      183
E   H   E   N   I   I   G   D   I   S   K   S   L   V   G   L   N

673                                                      720
ACACCACAGTACTTGATTTGCAGTTCAGTATTGAAACACTGAATGGCAGA
184                                                      199
T   T   V   L   D   L   Q   F   S   I   E   T   L   N   G   R

723                                                      770
GTCCAAGAGAATGCATTTAAACAACAAGAGGAGATGCGTAAATTAGAGGA
200                                                      216
V   Q   E   N   A   F   K   Q   Q   E   E   M   R   K   L   E   E

773                                                      820
GCGTATATACAATGCATCAGCAGAAATTAAGTCTCTAGATGAAAAACAAG
217                                                      233
R   I   Y   N   A   S   A   E   I   K   S   L   D   E   K   Q   V

823                                                      870
TATATTTGGAACAGGAAATAAAAGGGGAAATGAAACTGTTGAATAATATC
234                                                      249
Y   L   E   Q   E   I   K   G   E   M   K   L   L   N   N   I

873                                                      920
ACTAATGATCTGAGGCTGAAGGATTGGGAACATTCTCAGACATTGAAAAA
250                                                      266
T   N   D   L   R   L   K   D   W   E   H   S   Q   T   L   K   N

923                                                      970
TATCACTTTACTCCAAGGTCCTCCTGGACCTCCAGGTGAAAAAGGAGATA
267                                                      283
I   T   L   L   Q   G   P   P   G   P   P   G   E   K   G   D   R

973                                                      1020
GAGGCCCTCCTGGACAAAATGGTATACCAGGCTTTCCAGGTCTAATAGGT
284                                                      299
G   P   P   G   Q   N   G   I   P   G   F   P   G   L   I   G
```

FIG. 4B

```
1023                                                                       1070
ACTCCAGGTCTTAAAGGTGATCGGGGGATCTCTGGTTTACCTGGAGTTCG
300                                                                        316
T   P   G   L   K   G   D   R   G   I   S   G   L   P   G   V   R
```

```
1073                                                                       1120
AGGATTCCCAGGACCAATGGGGAAGACCGGGAAGCCAGGACTTAATGGAC
317                                                                        333
 G   F   P   G   P   M   G   K   T   G   K   P   G   L   N   G   Q
```

```
1123                                                                       1170
AAAAAGGCCAGAAGGGAGAAAAACCCACTCCAACCATCCAAACACAATCT
334                                                                        349
 K   G   Q   K   G   E   K   G   S   G   S   M   Q   R   Q   S
```

```
1173                                                                       1220
AATACAGTCCGACTGGTGGGTGGCAGCGGCCCTCACGAAGGCAGAGTGGA
350                                                                        366
N   T   V   R   L   V   G   G   S   G   P   H   E   G   R   V   E
```

```
1223                                                                       1270
GATTTTTCACGAAGGCCAGTGGGGTACGGTGTGTGACGACCGCTGGGAAC
367                                                                        383
 I   F   H   E   G   Q   W   G   T   V   C   D   D   R   W   E   L
```

```
1273                                                                       1320
TGCGTGGAGGACTGGTCGTCTGCAGGAGCTTGGGATACAAAGGTGTTCAA
384                                                                        399
 R   G   G   L   V   V   C   R   S   L   G   Y   K   G   V   Q
```

```
1323                                                                       1370
AGTGTGCATAAGCGAGCTTATTTTGGAAAAGGTACGGGTCCAATATGGCT
400                                                                        416
S   V   H   K   R   A   Y   F   G   K   G   T   G   P   I   W   L
```

```
1373                                                                       1420
GAATGAAGTATTTTGTTTCCCCAAAGAGTCATCCATTGAAGAGTGCAGAA
417                                                                        433
 N   E   V   F   C   F   G   K   E   S   S   I   E   E   C   R   I
```

```
1423                                                                       1470
TTAGACAGTGGGGTGTGAGAGCCTGTTCGCACGACGAAGATGCTGGGGTC
434                                                                        449
 R   Q   W   G   V   R   A   C   S   H   D   E   D   A   G   V
```
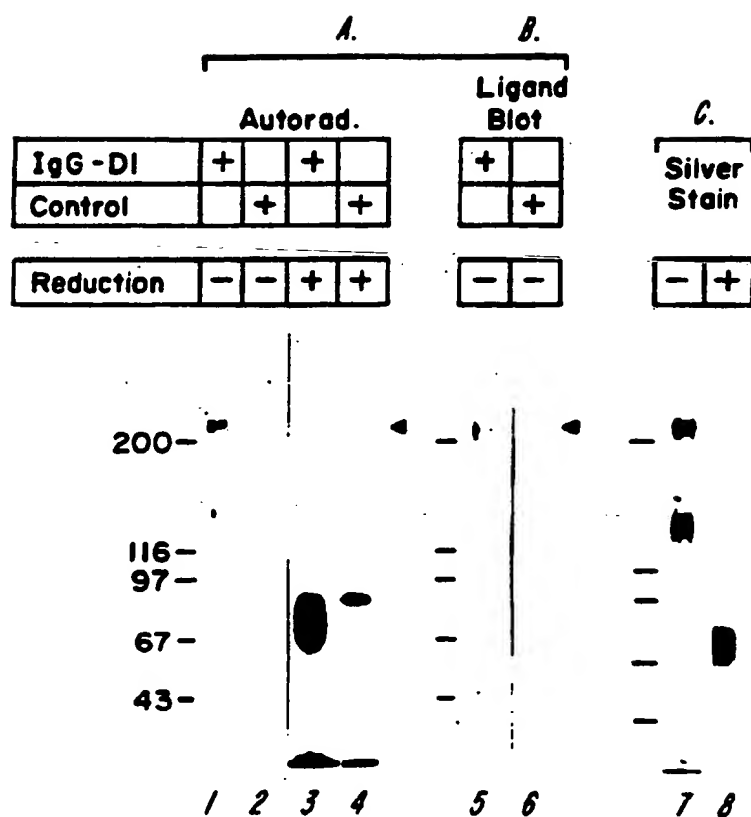
FIG. 4C

1473   1520
ACTTGCACTACATAATGCATCATATTTTCATTCACATTTTTTAAACTGTT
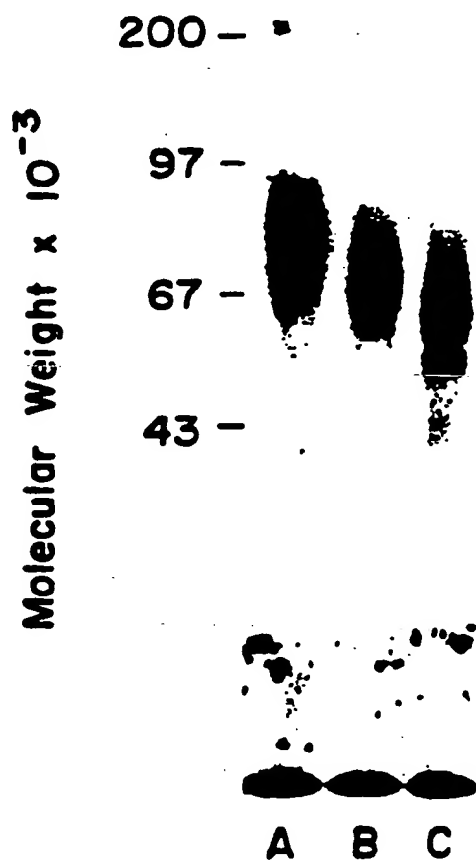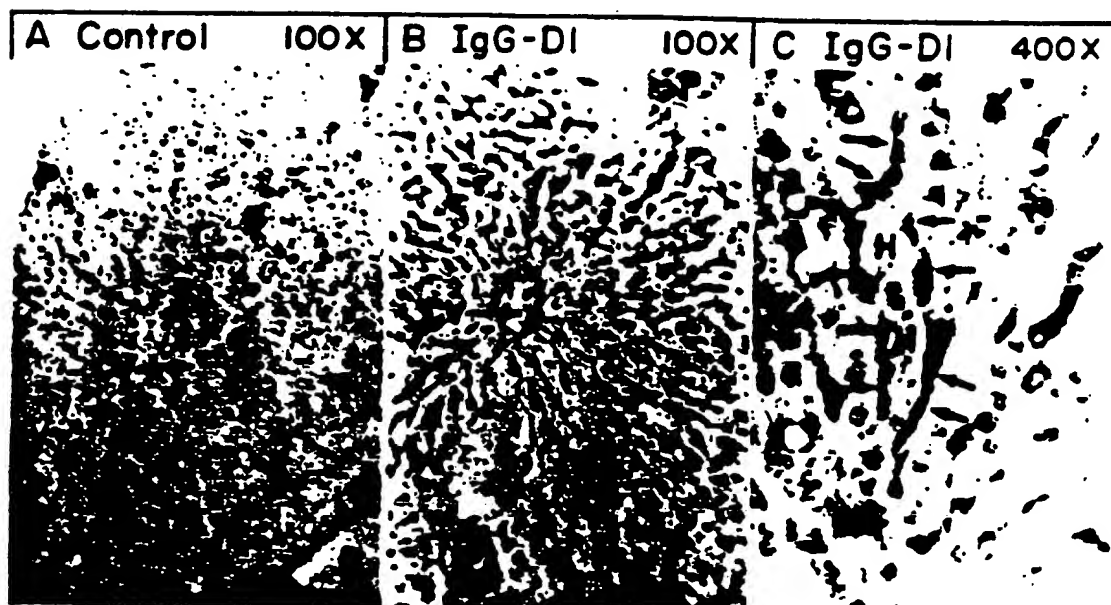450
T   C   T   T   *

1523   1570
ATAAAGTGATTTTTTTCCTTTGCTTCACTAAAATCAGCTTAATTAATATT
1573   1620
TAAGAAACTAAGAATTTTATCCACAGAAAAGGAATATTTAAAAATCACTG
1623   1670
GATAAACATATAAAATAGCTTCATATTTGCTTCAAATACCAGAACCATTT
1673
CAACTTCTGTAGGTTTTTAAGTGGCTCGTGCCGAATTC

FIG. 4D

FIG. 5

Sialidase	−	+	−
Endo F	−	−	+

200 −

Molecular Weight × 10^{-3}

97 −

67 −

43 −

A B C

FIG. 6

FIG. 7

A Control 100X	B IgG-DI 100X	C IgG-DI 400X

A. DiI-Ac-LDL-UPTAKE

B. IgG·DI IMMUNOBLOT

① DiI-Ac-LDL Alone

| Trypsin | − | + |

② With Excess Unlabeled Ac-LDL

Molecular Weight × 10^{-3}

200—

116—
97—
67—
43—

A   B

FIG. 8

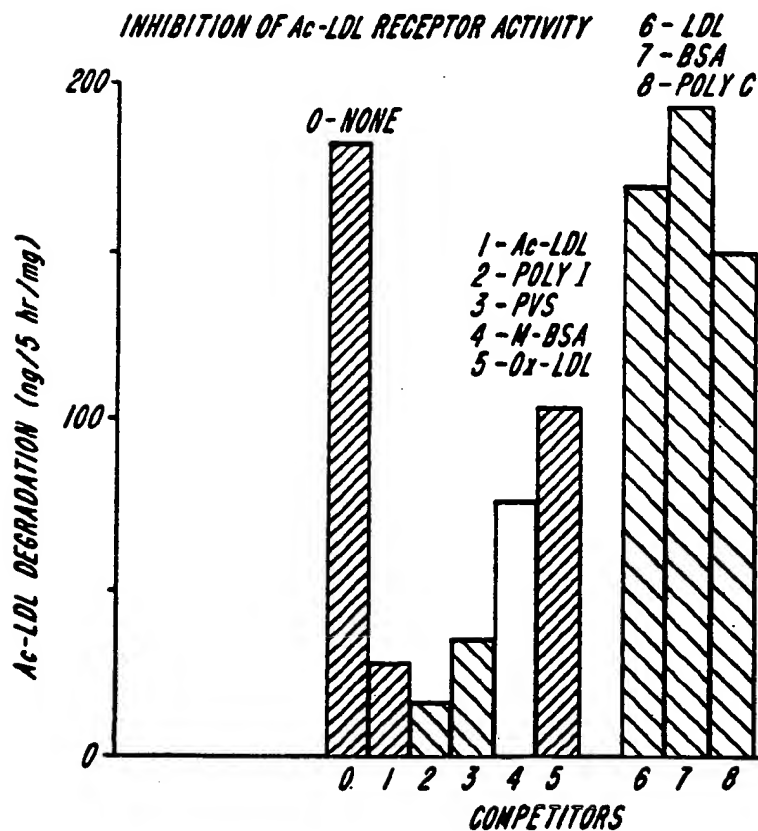| 200 nM PMA	−	+
Acetyl-LDL Degradation (ng/mg/5hr)	165	1347
Filter Binding Assay (ng/mg)	110	1044

Molecular Weight × 10^{-3}

200 —

116 —
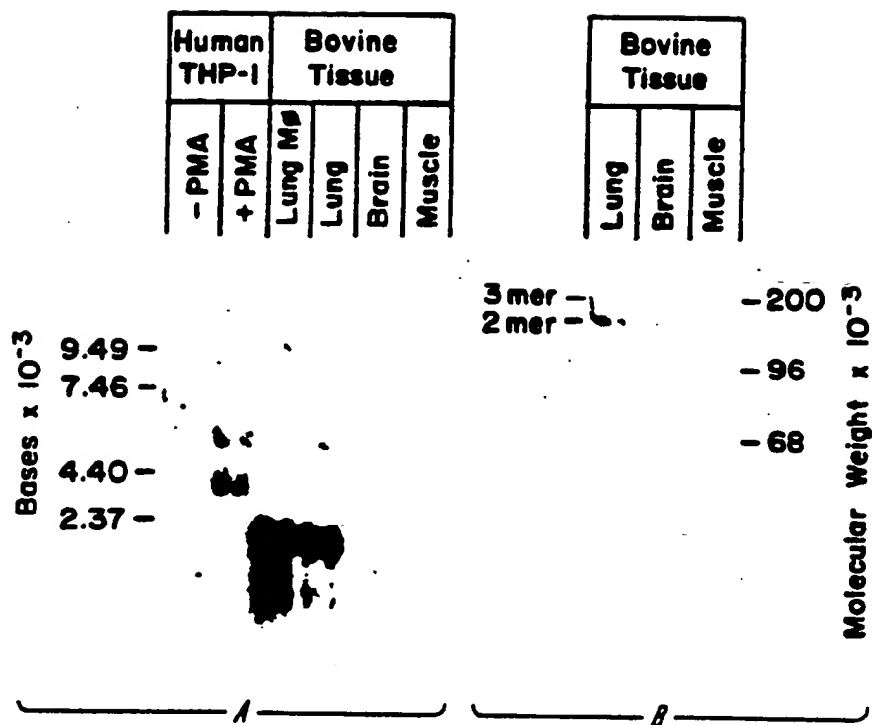97 —
67 —
43 —
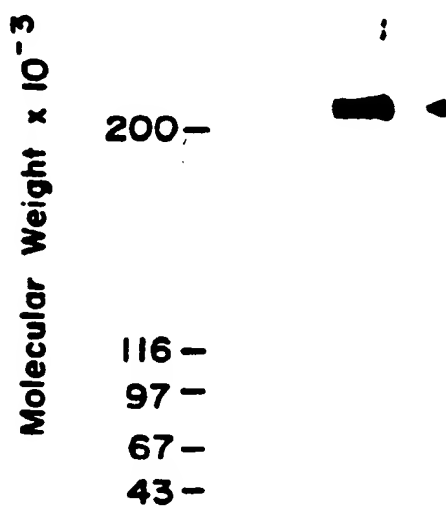
Ligand Blot

FIG. 9
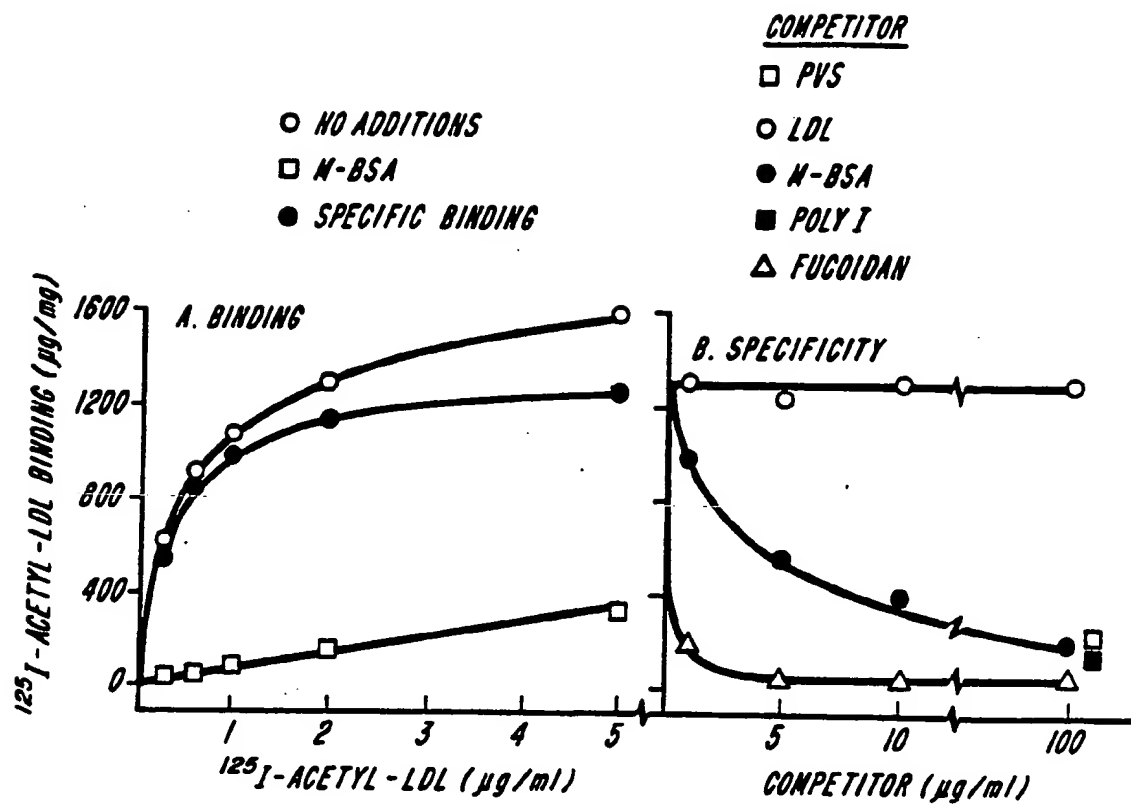
FIG. 10
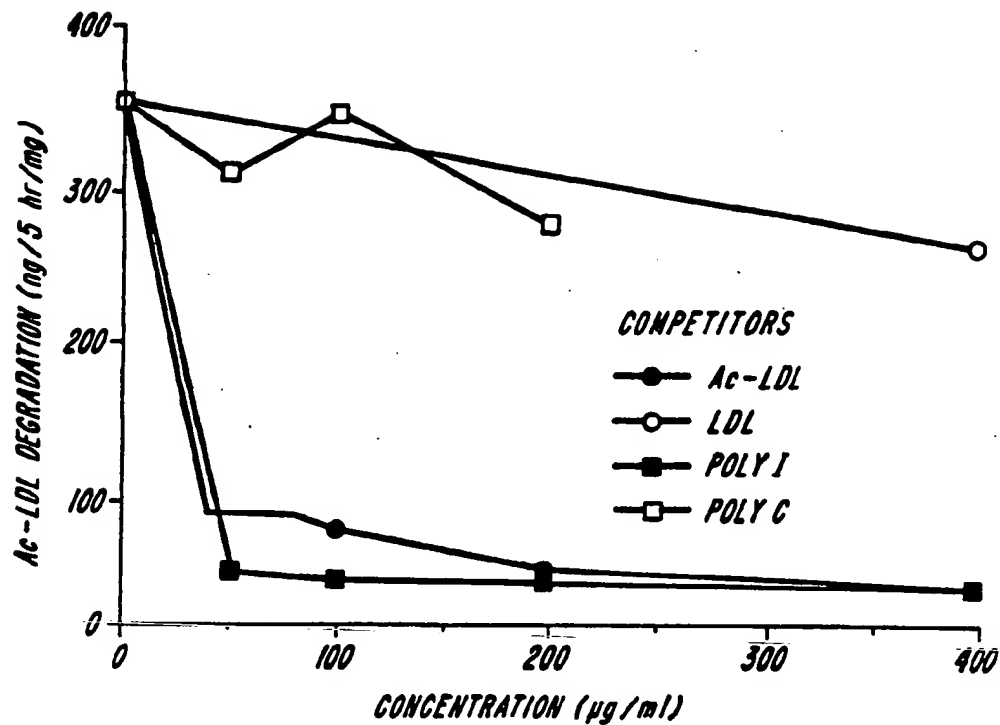
FIG. 11

FIG. 12

FIG. 13

-45

AGAGAAGTGGATAAATCAGTGCTGCTTTCTTTAGGACGAAAGAAGTATGGAGCA

                                           M E Q

10

GTGGGATCACTTTCACAATCAACAGGAGGACACTGATAGCTGCTCCGAATCTGT

 W D H F H N Q Q E D T D S C S E S V

64

GAAATTTGATGCTCGCTCAATGACAGCTTTGCTTCCTCCGAATCCTAAAAACAG

 K F D A R S M T A L L P P N P K N S

118

CCCTTCCCTTCAAGAGAAACTGAAGTCCTTCAAAGCTGCACTGATTGCCCTTTA

 P S L Q E K L K S F K A A L I A L Y

172

CCTCCTCGTGTTTGCAGTTCTCATCCCTCTCATTGGAATAGTGGCAGCTCAACT

 L L V F A V L I P L I G I V A A Q L

226

CCTGAAGTGGGAAACGAAGAATTGCTCAGTTAGTTCAACTAATGCAAATGATAT

 L K W E T K N C S V S S T N A N D I

280

AACTCAAAGTCTCACGGGAAAAGGAAATGACAGCGAAGAGGAAATGAGATTTCA

 T Q S L T G K G N D S E E E M R F Q

334

AGAAGTCTTTATGCAACACATGAGCAACATGGAGAAGAGAATCCAGCATATTTT

 E V F M E H M S N M E K R I Q H I L

388

AGACATGGAAGCCAACCTCATGGACACAGAGCATTTCCAAAATTTCAGCATGAC

 D M E A N L M D T E H F Q N F S M T

FIG. 14A

442

AACTGATCAAAGATTTAATGACATTCTTCTGCAGCTAAGTACCTTGTTTTCCTC
  T D Q R F N D I L L Q L S T L F S S

496

AGTCCAGGGACATGGGAATGCAATAGATGAAATCTCCAAGTCCTTAATAAGTTT
  V Q G H G N A I D E I S K S L I S L

550

GAATACCACATTGCTTGATTTGCAGCTCAACATAGAAAATCTGAATGGCAAAAT
  N T T L L D L Q L N I E N L N G K I

604

CCAAGAGAATACCTTCAAACAACAAGAGGAAATCAGTAAATTAGAGGAGCGTGT
  Q E N T F K Q Q E E I S K L E E R V

658

TTACAATGTATCAGCAGAAATTATGGCTATGAAAGAAGAACAAGTGCATTTGGA
  Y N V S A E I M A M K E E Q V H L E

712

ACAGGAAATAAAAGGAGAAGTGAAAGTACTGAATAACATCACTAATGATCTGAG
  Q E I K G E V K V L N N I T N D L R

766

ACTGAAAGATTGGGAACATTCTCAGACCTTGAGAAATATCACTTTAATTCAAGG
  L K D W E H S Q T L R N I T L I Q G

820

TCCTCCTGGACCCCCGGGTGAAAAAGGAGATCGAGGTCCCACTGGAGAAAGTGG
  P P G P P G E K G D R G P T G E S G

874

TCCACGAGGATTTCCAGGTCCAATAGGTCCTCCGGGTCTTAAAGGTGATCGGGG
  P R G F P G P I G P P G L K G D R G

FIG. 14B

928

AGCAATTGGCTTTCCTGGAAGTCGAGGACTCCCAGGATATGCCGGAAGGCCAGG

A I G F P G S R G L P G Y A G R P G

982

AAATTCTGGACCAAAAGGCCAGAAAGGGGAAAAGGGGAGTGGAAACACATTAAC

N S G P K G Q K G E K G S G N T L T

1036

TCCATTTACGAAAGTTCGACTGGTCGGTGGGAGCGGCCCTCACGAGGGGAGAGT

P F T K V R L V G G S G P H E G R V

1090

GGAGATACTCCACAGCGGCCAGTGGGGTACAATTTGTGACGATCGCTGGGAAGT

E I L H S G Q W G T I C D D R W E V

1144

GCGCGTTGGACAGGTCGTCTGTAGGAGCTTGGGATACCCAGGTGTTCAAGCCGT

R V G Q V V C R S L G Y P G V Q A V

1198

GCACAAGGCAGCTCACTTTGGACAAGGTACTGGTCCAATATGGCTGAATGAAGT

H K A A H F G Q G T G P I W L N E V

1252

GTTTTGTTTTGGGAGAGAATCATCTATTGAAGAATGTAAAATTCGGCAATGGGG

F C F G R E S S I E E C K I R Q W G

1306

GACAAGAGCCTGTTCACATTCTGAAGATGCTGGAGTCACTTGCACTTTATAATG

T R A C S H S E D A G V T C T L *

1360

CATCATATTTTCATTCACAACTATGAAATCGCTGCTCAAAAATGATTTTATTAC

FIG. 14C

1414

CTTGTTCCTGTAAAATCCATTTAATCAATATTTAAGAGATTAAGAATATTGCCC

1468

AAATAATATTTTAGATTACAGGATTAATATATTGAACACCTTCATGCTTACTAT

1522

TTTATGTCTATATTTAAATCATTTTAACTTCTATAGGTTTTTAAATGGAATTTT

1576

CTAATATAATGACTTATATGCTGAATTGAACATTTTGAAGTTTATAGCTTCCAG

1630

ATTACAAAGGCCAAGGGTAATAGAAATGCATACCAGTAATTGGCTCCAATTCAT

1684

AATATGTTCACCAGGAGATTACAATTTTTTGCTCTTCTTGTCTTTGTAATCTAT

1738

TTAGTTGATTTTAATTACTTTCTGAATAACGGAAGGGATCAGAAGATATCTTTT

1792

GTGCCTAGATTGCAAAATCTCCAATCCACACATATTGTTTTAAAATAAGAATGT

1846

TATCCAACTATTAAGATATCTCAATGTGCAATAACTTGTGTATTAGATATCAAT

1900

GTTAATGATATGTCTTGGCCACTATGGACCAGGGAGCTTATTTTTCTTGTCATG

1954

TACTGACAACTGTTTAATTGAATCATGAAGTAAATTGCC

FIG. 14D

INTERNATIONAL SEARCH REPORT

International Application No **PCT/US 89/05116**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 07 K 15/12, A 61 K 37/02, C 12 P 21/08, G 01 N 33/577,
G 01 N 33/92, C 12 N 15/12

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷	
Classification System ·	Classification Symbols
IPC⁵	G 01 N, A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	EP, A, 0189688 (NAKAGAMI) 6 August 1986 see the whole document cited in the application	1-18
P,X	Proc. Natl. Acad. Sci. USA, volume 85, December 1988, T. Kadoma et al.: "Purification and characterization of a bovine acetyl low density lipoprotein receptor", pages 9238-9242 see the whole article	1-13
Y		14-18
A	The Journal of Biological Chemistry, volume 260, no. 12, 25 June 1985, The American Society of Biological Chemists, Inc., (US), D.P. Via et al.: "Murine macrophage tumors are a source of a 260,000- Dalton acetyl-low density lipoprotein receptor", pages 7379-7386 see the whole article	1-18

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
9th March 1990	12. 04. 90

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	T.K. WILLIS

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 19-32 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:

Methods for treatment of the human or animal body by surgery
or therapy as well as diagnostic methods.

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers _____, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.   US 8905116
SA   32786

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 03/04/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0189688	06-08-86	JP-A- 61130238	18-06-86